

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE


Pre Application of:) Group Art Unit: 1648
ZEBEDEE et al.) Examining Attorney:
Serial No.: 10/677956) Zachariah Lucas
Filed: October 1, 2003) Date: February 13, 2006
Pasadena, California
For: METHODS AND SYSTEMS FOR)
PRODUCING RECOMBINANT)
VIRAL ANTIGENS)


EXHIBIT 2 TO THE
DECLARATION OF JOSEPH E. MUETH

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Attached hereto as Exhibit 2 to the Declaration of Joseph E. Mueth is United
States Patent Application Serial No. 08/819,857.

Date: February 13, 2006

Respectfully submitted,


Joseph E. Mueth
Registration No. 20,532

225 South Lake Avenue, 8th Floor
Pasadena, California 91101
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Facsimile: (626) 584-6862

THOMSON

FILE HISTORY

08/819,857

SERIAL NO: 08/819,857

COMPILED: 06 FEB 2006

DOCKET: 323-100

Class
Subclass
ISSUE CLASSIFICATION
SCANNED TO

ABANDONED

UTILITY SERIAL NUMBER	08/819857	PATENT DATE		PATENT NUMBER	
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SERIAL NUMBER	08/819,857	FILING DATE	03/17/97	CLASS	435	SUBCLASS	5	GROUP ART UNIT	1843	EXAMINER	Wortman
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APPLICANTS: SUZANNE ZEBEDEE, SAN DIEGO, CA; GENEVIEVE INCHAUPEPE, NEW YORK, NY;
MARC S. NASOFF, SAIN DIEGO, CA; ALFRED M. PRINCE, NEW YORK, NY

CONTINUING DATA***

VERIFIED THIS APPLN IS A CON OF 08/489,254 06/14/95 ABN
AND A CON OF 08/272,275 07/08/94 ABN
WHICH IS A CON OF 07/573,643 08/27/90 ABN

FOREIGN APPLICATIONS***

VERIFIED

FOREIGN FILING LICENSE GRANTED 05/01/97

Foreign priority claimed 35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED	STATE OR COUNTRY	SHEETS- DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
Verified and Acknowledged	Examiner's Initials	→	CA	4	9	1	\$770.00	2673/63066

ADDRESS: EDWARD P. GAMSON
WELSH AND KATZ LTD
22ND FLOOR
120 SOUTH RIVERSIDE PLAZA
CHICAGO IL 60606

TITLE: NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

U.S. DEPT. OF COMM./PAT. & TM - PTO-436L (Rev. 12-94)

PARTS OF APPLICATION FILED SEPARATELY		Applications Examiner	
NOTICE OF ALLOWANCE MAILED		CLAIMS ALLOWED	
		Total Claims	Print Claim
Assistant Examiner			
ISSUE FEE		DRAWING	
Amount Due	Date Paid	Sheets Drwg	Figs Drwg
			Print Fig
Label Area		ISSUE BATCH NUMBER	
Primary Examiner			
PREPARED FOR ISSUE			
<p>WARNING: The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 181 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only.</p>			

08/272275

Class	Subclass
ISSUE CLASSIFICATION	

UTILITY SERIAL NUMBER	08/272275	PATENT DATE		PATENT NUMBER	
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SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
08/272,275	07/08/94	435	5	1802	Wortman

APPLICANTS SUZANNE ZEBEDEE, SAN DIEGO, CA; GENEVIEVE INCHAUPE, NEW YORK, NY;
MARC S. NASOFF, SAN DIEGO, CA; ALFRED M. PRINCE, NEW YORK, NY.

CONTINUING DATA***

VERIFIED THIS APPLN IS A CON OF 07/573,643 08/27/90

FOREIGN/PCT APPLICATIONS***

VERIFIED

Foreign priority claimed 35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no <input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
Verified and Acknowledged	Examiner's Initials	→	CA	4	9	1	\$710.00	

ADDRESS WELSH & KATZ LTD
SUITE 1625
135 SOUTH LA SALLE STREET
CHICAGO IL 60603

TITLE NON-A, NON-B, HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

U.S. DEPT. of COM. Pat. & TM Office - PTO-435L (Rev. 10-78)

PARTS OF APPLICATION FILED SEPARATELY		Applications Examiner	
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		Total Claims	Print Claim
Assistant Examiner			
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Amount Due	Date Paid	Sheets Drwg.	Figs. Drwg.
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Label Area		ISSUE BATCH NUMBER	
Primary Examiner			
PREPARED FOR ISSUE			
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First to Processing and Review
DOE NASA
US COMM-DC

SERIAL NUMBER (Series of 1007) 07 573643	PATENT NUMBER				
SERIAL NUMBER 071573643	FILED DATE 08/27/90	CLASS 435	SUBCLASS 5	GROUP PART UNIT 182	EXAMINER Wortman

APPLICANTS SUZANNE ZEBEDEE, SAN DIEGO, CA; GENEVIEVE INCHAUSPE, NEW YORK, NY;
MARG S. NASOFF, SAN DIEGO, CA; ALFRED M. PRINCE, NEW YORK, NY.

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FOREIGN/PCT APPLICATIONS**
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Handwritten signature

Foreign priority claimed: 35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED →	STATE OR COUNTRY CA	SHEETS DRWGS. 4	TOTAL CLAIMS 40	INDEP. CLAIMS 7	FILING FEE RECEIVED \$1,402.00	ATTORNEY'S DOCKET NO. PHA0025P
Verified and Acknowledged		Examiner's Initials						

ADDRESS DRESSLER, GOLDSMITH, SHORE,
SUTKER & MILNAMOW, LTD.
11360 SORRENTO VALLEY RD., STE. 200 2 Prudential Plaza #4700
SAN DIEGO, CA 92121 Chicago, Ill 60601

TITLE NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES
U.S. DEPT. of COMM., Pat. & TM Office - PTO-436L (rev. 10-78)

PARTS OF APPLICATION FILED SEPARATELY				
NOTICE OF ALLOWANCE MAILED	PREPARED FOR ISSUE	CLAIMS ALLOWED		
Assistant Examiner		Docket Clerk	Total Claims	Print Claim
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Amount Due	Date Paid	Sheets Drwg.	Figs. Drwg.	Print Fig.
Primary Examiner				
ISSUE CLASSIFICATION		ISSUE BATCH NUMBER		
Class	Subclass			
Label Area				
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08/

Subclass	Class	ISSUE CLASSIFICATION

UTILITY SERIAL NUMBER	PATENT DATE	PATENT NUMBER
08/489254		

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
08/489,254	06/14/95	435	5	1802	Carthage

APPLICANTS: SUZANNE ZEBEDÉE, SAN DIEGO, CA; GENEVIEVE INCHAUSEPE, NEW YORK, NY;
MARC S. NASOFF, SAN DIEGO, CA; ALFRED M. PRINCE, NEW YORK, NY.

ABANDONED

CONTINUING DATA**
VERIFIED THIS APPLN IS A CON OF 08/272,275 07/08/94 ABN
WHICH IS A CON OF 07/573,643 08/27/90 ABN

FOREIGN/PCT APPLICATIONS**
VERIFIED

Foreign priority claimed 35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
Verified and Acknowledged	Examiner's Initials	→	CA	4	9	1	\$730.00	2678/63068

ADDRESS: EDWARD P. GAMSON,
WELSH AND KATZ,
435 SOUTH CASALLE STREET 120 South Riverside plaza
SUITE 1625 22nd floor
CHICAGO IL 60606

TITLE: NON-A, NON-B, HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

U.S. DEPT. OF COMM./PAT. & TM—PTO-436L (Rev. 12-8)

PARTS OF APPLICATION FILED SEPARATELY:		Applications Examiner	
NOTICE OF ALLOWANCE MAILED		CLAIMS ALLOWED	
Assistant Examiner		Total Claims	Print Claim
ISSUE FEE		DRAWING	
Amount Due	Date Paid	Sheets Drwg.	Figs. Drwg. Print Fig.
Label Area		ISSUE BATCH NUMBER	
Primary Examiner		PREPARED FOR ISSUE	
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CONTENTS

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1. Application <u>Pts</u> papers.	SEP 13 1990
2. 45 Day Letter (152)	
3. <u>10-8-90</u>	10-8-90
4. <u>10-27-90</u>	10-27-90
5. Statement (152)	Nov. 1, 1990 OK
6. <u>1-8-91</u>	1-8-91
7. <u>10/22/90</u>	10/22/90
8. <u>1/23/91</u>	1/23/91
9. <u>12-17-91</u>	12-17-91
10. <u>1/17/92</u>	1/17/92
11. <u>4/15/92</u>	4/15/92
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13. <u>7/20/92</u>	7/20/92
14. <u>10-05-92</u>	10-05-92
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20. <u>2-16-93</u>	2-16-93
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AUG 16 1994

GROUP 1800

1. Application papers:

29 Paul. L. L. L.

7/8/94

30 Disclosure Stmt

10/11/94

31 Reg. 3map

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32 Ext. of Terrie 3map

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JUL 28 1995

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Application papers.

GROUP 1800

1. *Rel Amtd D*

6-14-95

2. *Change of Address*

3/27/96

3. *Reflection*

9-17-96 9/16

4. *W. J. Edward*

5-13-97 5/12

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PATENT APPLICATION



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1. Application

papers

92538
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6/4/99

46 Request for Access

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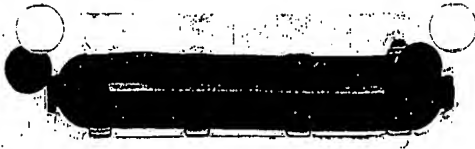
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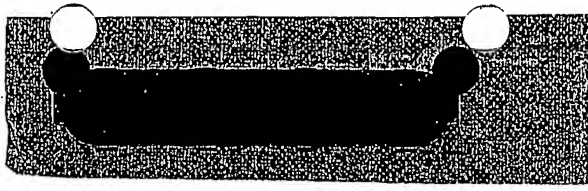
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436	828 828		
Updated		9/2/92	Dew
Updated		7/6/93	Dew

SEARCH NOTES		
	Date	Exmr.
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Updated	7/21/92	Dew
Updated APs	7/6/93	Dew

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Updated APs	9/21/98	D

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SEARCH NOTES		
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Reviewed parent case of 573643	12/8/94	Dew
Updated APS	12/9/94	Dew

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Class	Sub.	Date	Exmr.

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436	822 848	9/16/96	Bar
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SEARCH NOTES

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INTERFERENCE SEARCHED

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TYPIST	220	8-21-94
VERIFIER	332	8/4/94
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INDEX OF CLAIMS

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SYMBOLS

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- + Restricted
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- I Interference
- A Appeal
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TYPIST		
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EXAMINER	120	4-17
VERIFIER		11/27/90
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FILE MAINT.		

INDEX OF CLAIMS

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Not selected
Non-selected
Interference
Appealed
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VERIFIER	8	7/20/95
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PATENT APPLICATION SERIAL NO. 573643

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

100 TL 08/31/90 07523643

1 101 1,042.00 CK

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ABSTRACT

The present invention relates to a DNA segment encoding a recombinant non-A, non-B hepatitis structural protein or fusion protein and a recombinant DNA (rDNA) molecule capable of expressing either protein. Cells transformed with the rDNA, methods for producing the proteins in addition to compositions containing the proteins, and their use in diagnostic methods and systems, and in vaccines are also described.

1042 20 JUL 1994
EXPRESS MAIL LABEL NO. RB22138125

NON-A, NON-B HEPATITIS VIRUS ANTIGEN,
DIAGNOSTIC METHODS AND VACCINES

07 573643



Description

The present invention relates to a segment of deoxyribonucleic acid (DNA) that encodes a non-A, non-B hepatitis structural protein and a recombinant DNA (rDNA) that contains the DNA segment. Cells transformed with a rDNA of the present invention and methods for producing the NANBV structural protein are also contemplated. The invention also describes compositions containing the NANBV structural protein useful in diagnostic methods and in vaccines.

Background of the Invention

Non-A, non-B hepatitis (NANBH) is believed to be caused by a transmissible virus that has been referred to as both hepatitis C virus (HCV) and non-A, non-B hepatitis virus (NANBV). Although the transmissible disease was discovered years ago, a complete characterization of the causative agent is still being developed.

An isolate of NANBV has been obtained and portions of the viral genome were molecularly cloned and sequenced. Choo et al, Science, 244:359-362 (1989). Additional strains of NANBV were isolated and their genomes were partially characterized at the nucleotide sequence level. The similarities in nucleotide base sequence between these isolates of NANBV suggest that they are a part of a family of related viruses. Okamoto et al, Japan J. Exp. Med., 60:163-177 (1990). Properties of the NANBV genome suggest that NANBV may be a very distant relative of the flavivirus family. However, similarities in both

the size and hydropathicity of the structural proteins suggest that NANB viruses may also be distantly related to the pestivirus family. Miller et al, Proc. Natl. Acad. Sci., 87:2057-2061 (1990); and Okamoto et al, Japan J. Exp. Med., 60:163-177 (1990).

The difficulties in characterizing the NANBV isolates taxonomically, the lack of information regarding the proteins encoded by the NANBV genome, have made it difficult to identify relevant gene products useful for diagnostic markers and for producing NANBV vaccines.

The NANBV genome is comprised of a plus strand RNA molecule that codes for a single polyprotein. The gene products of NANBV are believed to include both structural and nonstructural proteins, based on homologies to characterized, related viruses. From these homologies, it is predicted that NANBV expresses a single polyprotein gene product from the complete viral genome, which is then cleaved into functionally distinct structural and nonstructural proteins. This type of viral morphogenesis precludes positive identification of the individual mature viral proteins until they have been physically isolated and characterized. Since no in vitro culturing system to propagate the virus has been developed for NANBV, no NANBV structural or nonstructural gene products (proteins) have been isolated from biological specimens or NANBV-infected cells. Thus, the identification of NANBV proteins, of their role in the viral life cycle, and of their role in disease, have yet to be determined. In particular, antigenic markers for NANBV-induced disease have yet to be fully characterized.

Only one NANBV gene product, namely the antigen C-100-3, derived from portions of the nonstructural

genes designated NS3 and NS4, has been expressed as a fusion protein and used to detect anti-C-100-3 antibodies in patients with various forms of NANB hepatitis. See, for example, Kuo et al, Science, 244:362-364 (1989); and International Application No. PCT/US88/04125. A diagnostic assay based on C-100-3 antigen is commercially available from Ortho Diagnostics, Inc. (Raritan, N.J.). This C-100-3 assay currently represents the state of the art in detecting NANBV infections. However, the C-100-3 antigen-based immunoassay has been reported to preferentially detect antibodies in sera from chronically infected patients. C-100-3 seroconversion generally occurs from four to six months after the onset of hepatitis, and in some cases C-100-3 fails to detect any antibody where an NANBV infection is present. Alter et al, New Eng. J. Med., 321:1538-39 (1989); Alter et al, New Eng. J. Med., 321:1494-1500 (1989); and Weiner et al, Lancet, 335:1-3 (1990). McFarlane et al, Lancet, 335:754-757 (1990), described false positive results when the C-100-3-based immunoassay was used to measure antibodies in patients with autoimmune chronic active hepatitis. In addition, Grey et al., Lancet, 335:609-610 (1990), describe false positive results using C-100-3-based immunoassay on sera from patients with liver disease caused by a variety of conditions other than NANBV.

A NANBV immunoassay that could accurately detect seroconversion at early times after infection, or that could identify an acute NANBV infection, is not presently available.

Summary of the Invention

The Hutchinson strain (Hutch) of non-A, non-B hepatitis virus (NANBV) has been propagated through passage in animals and portions of the virus have been

cloned and sequenced. Sequence data shows differences at both the nucleotide and amino acid level when compared to any previously reported NANBV strains. See, for comparison, Okamoto et al, Japan J. Exp. Med., 60:163-177 (1990); and International Application No. PCT/US88/04125.

The identified sequences have been shown herein to encode structural proteins of NANBV. The NANBV structural proteins are also shown herein to include antigenic epitopes useful for diagnosis of antibodies immunoreactive with structural proteins of NANBV, and for use in vaccines to induce neutralizing antibodies against NANBV.

The nucleotide sequence that codes for the amino terminal polyprotein portion of the structural genes of the Hutch strain of NANBV is shown in Figure 1. By comparison to putative relatives of NANBV, namely to other NANBV isolates, to flavivirus, and to pestivirus, the nucleotide sequence shown in Figure 1 is believed to encode structural proteins of NANBV, namely capsid and portions of envelope.

The structural antigens described herein are present in the putative capsid protein shown in Figure 1 from amino acid residue positions 1-120, and are present in the amino terminal portion of the putative envelope protein shown in Figure 1 from residue positions 121-326.

The present invention contemplates a DNA segment encoding a NANBV structural protein that comprises a NANBV structural antigen, preferably capsid antigen. A particularly preferred capsid antigen has an amino acid residue sequence represented by Figure 1 from residue 1 to residue 74. Preferably the DNA segment includes the nucleotide base sequence represented by Figure 1 from base position 1 to base position 222.

Also contemplated is a recombinant DNA molecule comprising a vector, preferably an expression vector, operatively linked to a DNA segment of the present invention. A preferred recombinant DNA molecule is
5 pGEX-3X-690:691, pGEX-3X-690:694, pGEX-3X-693:691, pGEX-3X-15:17, pGEX-3X-15:18, or pGEX-2T-15:17.

A NANBV structural protein is contemplated that comprises an amino acid residue sequence that defines a NANBV structural antigen, preferably a capsid
10 antigen, and more preferably one that includes the amino acid residue sequence shown in Figure 1 from residue 1 to residue 74. Fusion proteins comprised of a NANBV structural protein of this invention are also contemplated.

15 Further contemplated is a culture of cells transformed with a recombinant DNA molecule of this invention and methods of producing a NANBV structural protein of this invention using the culture.

Also contemplated is a composition comprising
20 NANBV structural protein. The composition is preferably characterized as being essentially free of (a) procaryotic antigens, and (b) other NANBV-related proteins.

Still further contemplated is a diagnostic system
25 in kit form comprising, in an amount sufficient to perform at least one assay, a NANBV structural protein composition of this invention, as a separately packaged reagent.

In another embodiment, the present invention
30 contemplates a diagnostic system, in kit form, comprising a fusion protein of this invention. Preferably, the diagnostic system contains the fusion protein affixed to a solid matrix.

Further contemplated is a method of assaying a
35 body fluid sample for the presence of antibodies

against at least one of the NANBV structural antigens described herein. The method comprises forming an immunoreaction admixture by admixing (contacting) the body fluid sample with a fusion protein of this invention. The immunoreaction admixture is maintained for a time period sufficient for any of the antibodies present to immunoreact with the fusion protein to form an immunoreaction product, which product, when detected, is indicative of the presence of anti-NANBV structural protein antibodies. Preferably, the fusion protein is affixed to a solid matrix when practicing the method.

In another embodiment, this invention contemplates a vaccine comprising an immunologically effective amount of a NANBV structural protein of this invention in a pharmaceutically acceptable carrier. The vaccine is essentially free of (a) procaryotic antigens, and (b) other NANBV-related proteins.

A prophylactic method for treating infection, which method comprises administering a vaccine of the present invention, is also contemplated.

Brief Summary of the Drawings

Figure 1 illustrates the nucleotide base sequence of a preferred DNA segment of the present invention that encodes portions of the structural proteins of the Hutch strain of NANBV. The base sequences are shown conventionally from left to right and in the direction of 5' terminus to 3' terminus using the single letter nucleotide base code (A=adenine, T=thymine, C=cytosine and G=guanine) with the position number of the first base residue in each row indicated to the left of the row showing the nucleotide base sequence.

The reading frame of the nucleotide sequence illustrated in Figure 1 is indicated by placement of the deduced amino acid residue sequence of the protein for which it codes below the nucleotide sequence such that the triple letter code for each amino acid residue (Table of Correspondence) is located directly below the three bases (codon) coding for each residue. The residue sequence is shown conventionally from left to right and in the direction of amino terminus to carboxy terminus. The position number for the last amino acid residue in each row is indicated to the right of the row showing the amino acid residue sequence.

Figure 2 illustrates the structure of a preferred fusion protein comprised of an amino-terminal polypeptide portion corresponding to residues 1-221 of glutathione-S-transferase, an intermediate polypeptide portion corresponding to residues 222-225 and defining a cleavage site for the protease Factor Xa, a linker portion corresponding to residues 226-234, a carboxy-terminal polypeptide portion corresponding to residues 235-308 defining a NANBV capsid antigen, and a carboxy-terminal linker portion corresponding to residues 309-315. Figure 2 also illustrates the nucleotide base sequence of a DNA segment that encodes the fusion protein illustrated therein. The nomenclature and presentation of sequence information is as described in Figure 1.

Detailed Description of the Invention

A. Definitions

Amino Acid: All amino acid residues identified herein are in the natural L-configuration. In keeping with standard polypeptide nomenclature, J. Biol. Chem., 243:3557-59, (1969), abbreviations for amino

acid residues are as shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

	<u>SYMBOL</u>		<u>AMINO ACID</u>
	<u>1-Letter</u>	<u>3-Letter</u>	
5	Y	Tyr	L-tyrosine
	G	Gly	glycine
	F	Phe	L-phenylalanine
	M	Met	L-methionine
10	A	Ala	L-alanine
	S	Ser	L-serine
	I	Ile	L-isoleucine
	L	Leu	L-leucine
	T	Thr	L-threonine
15	V	Val	L-valine
	P	Pro	L-proline
	K	Lys	L-lysine
	H	His	L-histidine
	Q	Gln	L-glutamine
20	E	Glu	L-glutamic acid
	W	Trp	L-tryptophan
	R	Arg	L-arginine
	D	Asp	L-aspartic acid
	N	Asn	L-asparagine
25	C	Cys	L-cysteine

It should be noted that all amino acid residue sequences, typically referred to herein as "residue sequences", are represented herein by formulae whose left to right orientation is in the conventional direction of amino-terminus to carboxy-terminus.

Antigen: A polypeptide or protein that is able to specifically bind to (immunoreact with) an antibody and form an immunoreaction product (immunocomplex). The site on the antigen with which

the antibody binds is referred to as an antigenic determinant or epitope.

Nucleotide: a monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via the glycosidic carbon (1' carbon of the pentose) and that combination of base and sugar is a nucleoside. When the nucleoside contains a phosphate group bonded to the 3' or 5' position of the pentose it is referred to as a nucleotide. A sequence of operatively linked nucleotides is typically referred to herein as a "base sequence", and is represented herein by a formula whose left to right orientation is in the conventional direction of 5'-terminus to 3'-terminus.

Base Pair (bp): A partnership of adenine (A) with thymine (T), or of cytosine (C) with guanine (G) in a double stranded DNA molecule.

B. DNA Segments

In living organisms, the amino acid residue sequence of a protein or polypeptide is directly related via the genetic code to the deoxyribonucleic acid (DNA) sequence of the structural gene that codes for the protein. Thus, a structural gene can be defined in terms of the amino acid residue sequence, i.e., protein or polypeptide, for which it codes.

An important and well known feature of the genetic code is its redundancy. That is, for most of the amino acids used to make proteins, more than one coding nucleotide triplet (codon) can code for or designate a particular amino acid residue. Therefore, a number of different nucleotide sequences may code for a particular amino acid residue sequence. Such nucleotide sequences are considered functionally

equivalent since they can result in the production of the same amino acid residue sequence in all organisms. Occasionally, a methylated variant of a purine or pyrimidine may be incorporated into a given nucleotide sequence. However, such methylations do not affect the coding relationship in any way.

In one embodiment the present invention contemplates an isolated DNA segment that comprises a nucleotide base sequence that encodes a NANBV structural protein comprising a NANBV structural antigen such as a capsid antigen, an envelope antigen, or both. Preferably, the structural antigen is immunologically related to the Hutch strain of NANBV.

More preferably, the encoded NANBV structural antigen has an amino acid residue sequence that corresponds, and preferably is identical, to the amino acid residue sequence shown in Figure 1.

In one embodiment, the putative capsid antigen includes an amino acid residue sequence shown in Figure 1 from residue 1 to residue 74. In another embodiment, the capsid antigen includes the sequence shown in Figure 1 from residue 69 to residue 120.

In another embodiment, the putative envelope antigen includes an amino acid residue sequence shown in Figure 1 from residue 121 to residue 176 or from residue 121 to residue 326.

Preferred DNA segments include a base sequence represented by the base sequence shown in Figure 1 from base position 1 to base position 222, from base position 205 to base position 360, from base position 361 to base position 528, or from base position 361 to base position 978.

In preferred embodiments, the length of the nucleotide base sequence is no more than about 3,000

bases, preferably no more than about 1,000, and more preferably no more than about 300 bases.

The amino acid residue sequence of a particularly preferred NANBV structural protein is
5 shown in Figure 2 from residue 1 to residue 316.

A purified DNA segment of this invention is substantially free of other nucleic acids that do not contain the nucleotide base sequences specified herein for a DNA segment of this invention, whether the DNA
10 segment is present in the form of a composition containing the purified DNA segment, or as a solution suspension or particulate formulation. By substantially free is means that the DNA segment is present as at least 10% of the total nucleic acid
15 present by weight, preferably greater than 50%, and more preferably greater than 90% of the total nucleic acid by weight.

In another embodiment, a DNA segment of this invention contains a nucleotide base sequence that
20 defines a structural gene capable of expressing a fusion protein. The phrase "fusion protein" refers to a protein having a polypeptide portion operatively linked by a peptide bond to a second polypeptide portion defining a NANBV structural antigen as
25 disclosed herein.

A preferred first polypeptide portion has an amino acid residue sequence corresponding to a sequence as shown in Figure 2 from about residue 1 to about residue 221, and is derived from the protein
30 glutathione-S-transferase (GST).

A preferred second polypeptide portion defining a NANBV structural antigen in a fusion protein includes an amino acid residue sequence represented by the sequence shown in Figure 1 from
35 residue 1 to residue 74, from residue 69 to residue

120, from residue 121 to residue 176, or from residue 121 to residue 326.

5 In one embodiment, a fusion protein can contain more than one polypeptide portion defining a NANBV structural antigen, as for example the combination of two polypeptide portions representing different structural antigens as shown by the sequence shown in Figure 1 from residue 1 to residue 120, or in Figure 1 from residue 1 to residue 326.

10 In particularly preferred embodiments, that portion of a fusion protein encoding DNA segment of this invention that codes for the polypeptide portion defining a NANBV capsid antigen includes a nucleotide base sequence corresponding to a sequence that codes for an amino acid residue sequence as shown in Figure 15 1 from residue 1 to residue 74, and more preferably includes a nucleotide base sequence corresponding to a base sequence as shown in Figure 1 from base 1 to base 222.

20 In another embodiment, that portion of a fusion protein encoding DNA segment of this invention that codes for the polypeptide portion defining a NANBV envelope antigen includes a nucleotide base sequence corresponding to a sequence that codes for an amino acid residue sequence as shown in Figure 1 25 from residue 121 to residue 176 or from residue 121 to residue 326, and more preferably includes a nucleotide base segment corresponding in base sequence to the sequence shown in Figure 1 from base 361 to base 528 or from base 361 to base 978, respectively.

30 A particularly preferred fusion protein encoding DNA segment of this invention has a nucleotide base sequence corresponding to the sequence shown in Figure 2 from base 1 to base 945.

In preferred embodiments, a DNA segment of the present invention is bound to a complimentary DNA segment, thereby forming a double stranded DNA segment. In addition, it should be noted that a double stranded DNA segment of this invention preferably has a single stranded cohesive tail at one or both of its termini.

A DNA segment of the present invention can easily be prepared from isolated virus obtained from the blood of a NANBV-infected individual such as described herein or can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci et al., J. Am. Chem. Soc., 103:3185 (1981). (The disclosures of the art cited herein are incorporated herein by reference.) Of course, by chemically synthesizing the structural gene portion, any desired modifications can be made simply by substituting the appropriate bases for those encoding a native amino acid residue. However, DNA segments including sequences identical to a segment shown in Figures 1 or 2 are preferred.

In addition, a DNA segment can be prepared by first synthesizing oligonucleonucleotides that correspond to portions of the DNA segment, which oligonucleotides are then assembled by hybridization and ligation into a complete DNA segment. Such methods are also well known in the art. See for example, Paterson et al., Cell, 48:441-452 (1987); and Lindley et al., Proc. Natl. Acad. Sci., 85:9199-9203 (1988), where a recombinant peptide, neutrophil-activated factor, was produced from the expression of a chemically synthesized gene in E. coli.

C. Recombinant DNA Molecules

The present invention further contemplates a recombinant DNA (rDNA) that includes a DNA segment of the present invention operatively linked to a vector. A preferred rDNA of the present invention is

5 characterized as being capable of directly expressing, in a compatible host, a NANBV structural protein or fusion protein of this invention. Preferred DNA segments for use in a rDNA are those described herein above.

10 By "directly expressing" is meant that the mature polypeptide chain of the protein is formed by translation alone as opposed to proteolytic cleavage of two or more terminal amino acid residues from a larger translated precursor protein. Preferred rDNAs
15 of the present invention are the plasmids pGEX-3X-690:694, pGEX-3X-693:691, pGEX-3X-690:691, pGEX-3X-15:17, pGEX-3x-15:18, and pGEX-2T-15:17 described in Example 1.

20 A recombinant DNA molecule of the present invention can be produced by operatively linking a vector to a DNA segment of the present invention. Exemplary rDNA molecules and the methods for their preparation are described in Example 1.

25 As used herein, the term "vector" refers to a DNA molecule capable of autonomous replication in a cell and to which another DNA segment can be operatively linked so as to bring about replication of the attached segment. Typical vectors are plasmids, bacteriophage and the like. Vectors capable of
30 directing the expression of a NANBV structural protein or fusion protein are referred to herein as "expression vectors". Thus, a recombinant DNA molecule (rDNA) is a hybrid DNA molecule comprising at least two nucleotide sequences not normally found
35 together in nature.

The choice of vector to which a DNA segment of the present invention is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein
5 expression, and the host cell to be transformed, these being limitations inherent in the art of constructing recombinant DNA molecules. However, a vector contemplated by the present invention is at least capable of directing the replication, and preferably
10 also expression, of the recombinant or fusion protein structural gene included in DNA segments to which it is operatively linked.

In preferred embodiments, a vector contemplated by the present invention includes a
15 procaryotic replicon (ori), i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extrachromosomally in a procaryotic host cell, such as a bacterial host cell, transformed therewith. Such
20 replicons are well known in the art. In addition, those embodiments that include a procaryotic replicon also typically include a gene whose expression confers drug resistance to a bacterial host transformed therewith. Typical bacterial drug resistance genes
25 for use in these vectors are those that confer resistance to ampicillin or tetracycline. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA).

Those vectors that include a procaryotic
30 replicon can also include a procaryotic promoter capable of directing the expression (transcription and translation) of the gene encoding a NANBV structural protein or fusion protein in a bacterial host cell, such as E. coli, transformed therewith. A promoter is
35 an expression control element formed by a DNA sequence

that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for
5 insertion of a DNA segment of the present invention. A typical vector is pPL-lambda available from Pharmacia, (Piscataway, N.J.).

Vector plasmids having a bacterial promoter that is inducible with IPTG are the pTTQ plasmids
10 available from Amersham (Arlington Heights, IL), and the pKK223-3 plasmid available from Pharmacia. Additional expression vectors for producing in procaryotes a cloned gene product in the form of a fusion protein are well known and commercially
15 available.

Although the expression vectors pGEX-3X and pGEX-2T have been used as exemplary in producing the fusion proteins described herein, other functionally equivalent expression vectors can be used.

Functionally equivalent vectors contain an expression promoter that is inducible by IPTG for fusion protein expression in E. coli, and a configuration such that upon insertion of the DNA segment into the vector a fusion protein is produced. A commercially available
25 vectors functionally equivalent to pGEX-3X and pGEX-2T is the pGEMEX-1 plasmid vector from Promega (Madison, WI) that produces a fusion between the amino terminal portion of the T7 gene 10 protein and the cloned insert gene, and the pGEX-3X and pGEX-2T plasmids from
30 Pharmacia that produce a fusion with the enzyme glutathione-s-transferase (GST).

The construction and use of the pGEX-3X and pGEX-2T vectors have been described by Smith et al., Gene, 67:31-40 (1988), which reference is hereby
35 incorporated by reference.

In particularly preferred embodiments, a fusion protein contains a GST derived polypeptide-portion as an added functional domain operatively linked to a NANBV structural antigen of this invention. Any inducible promoter drive vector, such as the vectors pTTQ, pKK223-3, pGEX-3X or pGEX-2T described above and the like, can be used to express a GST-NANBV structural protein, referred to herein as a GST:NANBV fusion protein. Thus, although the pGEX-3X and pGEX-2T vectors are described as exemplary, the DNA molecules of this invention are not to be construed as limited to these vectors, because the invention in one embodiment is directed to an rDNA for expression of a protein having NANBV structural antigens fused to GST and not drawn to the vector per se.

A variety of methods have been developed to operatively link DNA segments to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted and to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. A DNA segment generated by endonuclease restriction digestion is treated with bacteriophage T4 DNA polymerase or E. coli DNA polymerase I, enzymes that remove protruding, 3', single-stranded termini with their 3'-5' exonucleolytic activities and fill in recessed 3' ends with their polymerizing activities. The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker

molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies, Inc., New Haven, CN.

Also contemplated by the present invention are RNA equivalents of the above described recombinant DNA molecules.

D. Transformed Cells and Cultures

The present invention also relates to a procaryotic host cell transformed with a recombinant DNA molecule of the present invention. Preferred rDNA molecules for use in a transformed cell are those described herein above and preferably are rDNA's capable of expressing a recombinant or fusion protein. Specific preferred embodiments of transformed cells are those which contain an rDNA molecule having one of the preferred DNA segments described herein above, and particularly cells transformed with the rDNA plasmid pGEX-3X-690:694, pGEX-3X-693:691, pGEX-3X-690:691, pGEX-3X-15:17, pGEX-3X-15:18 or pGEX-2T-15:17.

Bacterial cells are preferred procaryotic host cells and typically are a strain of E. coli, such as, for example, the E. coli strain DH5 available from Bethesda Research Laboratories, Inc., Bethesda, MD. Transformation of appropriate cell hosts with a recombinant DNA molecule of the present invention is

accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of procaryotic host cells, see, for example, Cohen et al., Proc. Natl. Acad. Sci. USA, 69:2110 (1972); and Maniatis et al., Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989).

Successfully transformed cells, i.e., cells that contain a recombinant DNA molecule of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an rDNA of the present invention can be isolated as single colonies. Cells from those colonies can be harvested, lysed and their DNA content examined for the presence of the rDNA using a method such as that described by Southern, J. Mol. Biol., 98:503 (1975) or Berent et al., Biotech., 3:208 (1985).

In addition to directly assaying for the presence of rDNA, cells transformed with the appropriate rDNA can be identified by well known immunological methods when the rDNA is capable of directing the expression of a NANBV structural protein. For example, cells successfully transformed with an expression vector of this invention produce proteins displaying NANBV structural protein antigenicity. Samples of cells suspected of being transformed are harvested and assayed for the presence of a NANBV structural antigen using antibodies specific for that antigen, such antibodies being described further herein.

Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a

nutrient medium. Preferably, the culture also contains a protein displaying NANBV structural protein antigenicity.

5 Nutrient media useful for culturing transformed host cells are well known in the art and can be obtained from several commercial sources.

E. Methods for Producing NANBV structural proteins and Fusion Proteins

10 Another aspect of the present invention pertains to a method for producing recombinant proteins and fusion proteins of this invention.

15 The present method entails initiating a culture comprising a nutrient medium containing host cells, preferably E. coli cells, transformed with a recombinant DNA molecule of the present invention that is capable of expressing a NANBV structural protein or a fusion protein. The culture is maintained for a time period sufficient for the transformed cells to
20 express the NANBV structural protein or fusion protein. The expressed protein is then recovered from the culture.

25 Expression vectors and expression vector culturing conditions for producing NANBV structural proteins are generally well known in the art. Such vectors and culturing conditions can be altered without affecting the spirit of the present invention. However, preferred are the vectors designed specifically for the production of proteins not
30 normally found in the host cell used to express a NANBV structural protein. Exemplary are the vectors that contain inducible promoters for directing the expression of DNA segments that encode the NANBV structural protein. Vectors with promoters inducible
35 by IPTG are also well known. See for example plasmids

PTTQ and pKK223-3 available from Amersham and Pharmacia respectively. Particularly preferred are the promoters inducible by IPTG present in the pGEX vectors pGEX-3X and pGEX-2T described herein.

5 Using vectors with inducible promoters, expression of NANBV structural proteins requires an induction phase at the beginning of the above described maintenance step for expressing the protein, as is known and described in detail in Example 2.

10 Methods for recovering an expressed protein from a culture are well known in the art and include fractionation of the protein-containing portion of the culture using well known biochemical techniques. For instance, the methods of gel filtration, gel
15 chromatography, ultrafiltration, electrophoresis, ion exchange, affinity chromatography and the like, such as are known for protein fractionations, can be used to isolate the expressed proteins found in the culture. In addition, immunochemical methods, such as
20 immunoaffinity, immunoadsorption and the like can be performed using well known methods.

 Particularly preferred are isolation methods that utilize the presence of the polypeptide portion defining glutathione-S-transferase (GST) as a means to
25 separate the fusion protein from complex mixtures of protein. Affinity adsorption of a GST-containing fusion protein to a solid phase containing glutathione affixed thereto can be accomplished as described by Smith et al., Gene, 67:31 (1988). Alternatively, the
30 GST-containing polypeptide portion of the fusion protein can be separated from the NANBV structural antigen by selective cleavage of the fusion protein at the factor Xa cleavage site, according to the methods of Smith et al., Gene, 67:31 (1988). Exemplary
35 isolation methods are described in Examples 5 and 6.

In addition to its preparation by the use of a rDNA expression vector, a NANBV structural protein comprising a NANBV structural antigen can be prepared in the form of a synthetic polypeptide. Polypeptides can be synthesized by any of the techniques that are known to those skilled in the polypeptide art. Synthetic chemistry techniques, such as a solid-phase Merrifield-type synthesis, are preferred for reasons of purity, antigenic specificity, freedom from undesired side products, ease of production and the like, and can be carried out according to the methods described in Merrifield et al., J. Am. Chem. Soc., 85:2149-2154 (1963) and Houghten et al., Int. J. Pept. Prot. Res., 16:311-320 (1980). An excellent summary of the many techniques available can be found in J.M. Steward and J.D. Young, "Solid Phase Peptide Synthesis", W.H. Freeman Co., San Francisco, 1969; M. Bodanszky, et al., "Peptide Synthesis", John Wiley & Sons, Second Edition, 1976; and J. Meienhofer, "Hormonal Proteins and Peptides", Vol. 2, p. 46, Academic Press (New York), 1983, for solid phase peptide synthesis, and E. Schroder and K. Kubke, "The peptides", Vol. 1, Academic Press (New York), 1965, for classical solution synthesis, each of which is incorporated herein by reference.

F. NANBV structural protein and Fusion Protein Compositions

In another embodiment, the present invention contemplates a composition containing an isolated NANBV structural protein comprising an amino acid residue sequence that defines a NANBV structural antigen of this invention.

By isolated is meant that a NANBV structural protein of this invention is present in a composition

as a major protein constituent, typically in amounts greater than 10% of the total protein in the composition, but preferably is greater than 90% of the total protein in the composition.

5 A NANBV structural antigen, as used herein, is a structural protein coded by the genome of NANBV and has the properties of an antigen as defined herein, namely, to be able to immunoreact specifically with an antibody. NANBV structural proteins have been
10 tentatively designated as capsid and envelope, and have been partially characterized as described herein to contain the NANBV structural antigens capsid and envelope, respectively.

15 NANBV capsid antigen comprises an amino acid residue sequence that is immunologically related in sequence to the putative Hutch strain NANBV capsid antigen, whose sequence is shown in Figure 1 from residue 1 to residue 120.

20 NANBV envelope antigen comprises an amino acid residue sequence that is immunologically related in sequence to the putative Hutch strain NANBV envelope antigen, a portion of whose sequence is shown in Figure 1 from residue 121 to residue 326.

25 By "immunologically related" is meant that sufficient homology in amino acid sequence is present in the two protein sequences being compared that antibodies specific for one protein immunoreact (crossreact) with the other protein. Immunological crossreactivity can be measured by methods well known
30 including the immunoassay methods described herein.

35 As used herein, the phrase "recombinant protein" refers to a protein of at least 20 amino acid residues in length, and preferably at least 50 residues, that includes an amino acid residue sequence that corresponds, and preferably is identical, to a

portion of the NANBV structural protein shown in Figure 1.

In one embodiment a NANBV structural protein includes an amino acid residue sequence that is immunologically related to, and preferably is identical to, the sequence shown in Figure 1 from residue 1 to residue 74. This NANBV structural protein is particularly preferred for use in diagnostic methods and systems because the capsid antigen contained therein was demonstrated herein to be particularly useful in detecting acute NANBV infection. Related NANBV structural proteins include a sequence shown in Figure 1 from residue 1 to residue 120, from residue 1 to residue 176, and from residue 1 to residue 315. Exemplary are the proteins described herein having a sequence shown in Figure 2 from residue 1 to residue 316, or having the sequence of the expressed protein coded for by the rDNA plasmid pGEX-3X-690:691.

In another embodiment a NANBV structural protein includes an amino acid residue sequence that is immunologically related to, and preferably is identical to, the sequence shown in Figure 1 from residue 69 to residue 120. An exemplary NANBV structural protein has the sequence of the expressed protein coded for by the rDNA plasmid pGEX-3X-693:691.

Additional NANBV structural proteins containing NANBV envelope antigen are contemplated that include an amino acid residue sequence that is immunologically related to, and preferably is identical to, the sequence shown in Figure 1 from residue 121 to residue 176. Exemplary are the proteins having a sequence of the expressed protein coded for by one of the rDNA plasmids pGEX-3X-15:17, pGEX-3X-15:18 and pGEX-2T-15:17.

In preferred embodiments a NANBV structural protein is essentially free of both procaryotic antigens (i.e., host cell-specific antigens) and other NANBV-related proteins. By "essentially free" is
5 meant that the ratio of NANBV structural antigen to either procaryotic antigen or other NANBV-related protein is at least 10:1, preferably is 100:1, and more preferably is 200:1.

The presence and amount of contaminating
10 protein in a NANBV structural protein preparation can be determined by well known methods. Preferably, a sample of the composition is subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to separate the NANBV structural protein
15 from any protein contaminants present. The ratio of the amounts of the proteins present in the sample is then determined by densitometric soft laser scanning, as is well known in the art. See Guilian et al., Anal. Biochem., 129:277-287 (1983).

20 A NANBV structural protein can be prepared as an isolated protein, and more preferably essentially free of procaryotic antigens or NANBV non-structural antigens by the methods disclosed herein for producing NANBV structural proteins. Particularly
25 preferred are methods which rely on the properties of a polypeptide region of a fusion protein, which region is present in the fusion protein to facilitate separation of the fusion protein from host cell proteins on the basis of affinity. Exemplary is the
30 GST-containing fusion protein shown in Figure 2 wherein the GST polypeptide region provides the fusion protein (with a functional domain) having an affinity to bind to the normal substrate for GST, namely glutathione. The purification of a fusion protein

having a GST polypeptide region is described further herein.

In a related embodiment, a composition comprising an isolated fusion protein is also contemplated by the present invention that comprises a NANBV structural protein of this invention operatively linked at one or both termini to another polypeptide by a peptide bond. The added polypeptide can be any polypeptide designed to increase the functional domains present on the fusion protein. The added functional domains are included to provide additional immunogenic epitopes, to add mass to the fusion protein, to alter the solubility of the fusion protein, to provide a means for affinity-based isolation of the fusion protein, and the like. Exemplary added functional domains are the Thrombin or Factor Xa specific cleavage sites provided when a subject fusion protein is produced in the vector pGEX-3X or pGEX-2T, respectively, as described herein. An additional exemplary domain is the GST-derived protein domain that allows rapid isolation using affinity chromatography to a solid phase containing glutathione affixed thereto.

A fusion protein of the present invention includes an amino acid residue sequence corresponding from its amino-terminus to its carboxy-terminus to the amino acid residue sequence shown in Figure 1 from residue 1 to residue 74, from residue 69 to residue 120, from residue 121 to residue 176, or from residue 121 to residue 326. A preferred fusion protein has a sequence corresponding to, and more preferably identical to, the residue sequence in Figure 2 from residue 1 to residue 315. Other preferred fusion proteins are defined by the amino acid residue sequence of the expressed protein coding sequence

present in the rDNA plasmids pGEX-3X-690:691, pGEX-3X-693:691, pGEX-3X-15:17, pGEX-3X-15:18 and pGEX-2T-15:17.

5 The phrase "fusion protein", when used herein refers to an isolated protein as it was defined for a NANBV structural protein of this invention. Thus an isolated fusion protein is a composition having a fusion protein of this invention in amounts greater than 10 percent of the total protein in the
10 composition, and preferably greater than 90 percent of the total protein in the composition.

15 A preferred fusion protein is a heterologous fusion protein, that is, a fusion protein that contains a polypeptide portion derived from a protein originating in a heterologous species of virus, organism, pathogen or animal, ie, a non-NANBV protein. Preferably a heterologous fusion protein contains a non-NANBV polypeptide portion that is not
20 immunologically related to a NANBV structural antigen of this invention.

25 In one embodiment, a fusion protein contains a functional domain that provides an immunogenic or antigenic epitope other than the NANBV structural antigen defined herein and is preferably derived from a separate pathogen, or from several pathogens. The functional domain is immunogenic where that domain is present to form a polyvalent vaccine or immunogen for the purpose of inducing antibodies immunoreactive with both NANBV structural protein and a second pathogen.
30 The functional domain is antigenic where that domain is present to form a polyvalent antigen for use in diagnostic systems and methods for detecting at least two species of antibodies.

35 Of particular interest in this embodiment are fusion proteins designed to include a functional

domain that is derived from other hepatitis-causing viruses, such as Hepatitis B virus, and Hepatitis A virus. These viruses have been well characterized to contain antigenic determinants and immunogenic determinants suitable for use in the fusion protein of this invention, and provide the advantage of multipurpose biochemical reagents in both diagnostic and vaccine applications. Additionally, the included functional domain can contain amino acid sequences from other pathogens, preferably those which may also infect individuals with NANBV hepatitis, such as HIV.

Preferred NANBV structural proteins or fusion proteins comprising a NANBV structural antigen of the present invention are in non-reduced form, i.e., are substantially free of sulfhydryl groups because of intramolecular Cys-Cys bonding.

In preferred compositions, the NANBV structural protein or fusion protein as described herein, is present, for example, in liquid compositions such as sterile suspensions or solutions, or as isotonic preparations containing suitable preservatives.

One such composition useful for inducing anti-NANBV structural protein antibodies in a mammal is referred to as a vaccine and contains a NANBV structural protein or fusion protein of this invention.

G. Vaccines

1. Introduction

The word "vaccine" in its various grammatical forms is used herein to describe a type of inoculum containing one or more NANBV structural antigens of this invention as an active ingredient

that is used to induce active immunity in a host mammal against NANBV.

A vaccine comprises, as an active immunogenic ingredient, a NANBV structural protein or fusion protein of this invention.

Because a vaccine is typically designed to induce specific antibodies, it is preferred that a vaccine contain a NANBV structural protein comprised of only NANBV structural antigens and not other functional domains as described for a fusion protein. Thus a preferred vaccine contains a NANBV structural protein of this invention that includes an amino acid residue sequence shown in Figure 1 from residue 1 to residue 74, from residue 69 to residue 120, from residue 121 to residue 176, or from residue 121 to residue 326. Particularly preferred as an active ingredient in a vaccine is a NANBV structural protein having the amino acid residue sequence shown in Figure 1 from residue 1 to residue 74, from residue 1 to residue 120, or shown in Figure 2 from residue 226 to residue 315.

Alternatively, a polyvalent vaccine is contemplated that comprises a fusion protein that has two immunogenic functional domains and is useful to induce two classes of antibodies each specific for a different antigen; namely a first NANBV structural antigen as described herein, and a second antigen present on a distinct pathogen. Preferred second antigens are derived from pathogens that are typically found in association with NANBV-infected patients, namely Hepatitis B Virus, Human Immunodeficiency Virus (HIV) and the like.

2. Preparation

The preparation of a vaccine that contains a protein or polypeptide as an active ingredient is well understood in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation can also be emulsified.

The active immunogenic ingredient is dissolved, dispersed or admixed in an excipient that is pharmaceutically acceptable and compatible with the active ingredient as is well known. The phrases "suitable for human use" and "pharmaceutically acceptable" (physiologically tolerable) refer to molecular entities and compositions that typically do not produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. Suitable excipients may take a wide variety of forms depending on the intended use and are, for example, aqueous solutions containing saline, phosphate buffered saline (PBS), dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, mineral oils, carriers or adjuvants which enhance the effectiveness of the vaccine. A preferred embodiment contains at least about 0.01% to about 99% of NANBV structural protein or fusion as an active ingredient, typically at a concentration of about 10 to 200 ug-of active ingredient per ml of excipient.

3. Carriers

One or more additional amino acid residues may be added to the amino- or carboxy-termini of the

NANBV structural protein to assist in binding the protein to a carrier if not already present on the protein. Cysteine residues added at the amino- or carboxy-termini of the protein have been found to be particularly useful for forming polymers via disulfide bonds. However, other methods well known in the art for preparing conjugates can also be used. Exemplary additional linking procedures include the use of Michael addition reaction products, dialdehydes such as glutaraldehyde, Klipstein et al., J. Infect. Dis., 147:318-326 (1983) and the like, or the use of carbodiimide technology as in the use of a water-soluble carbodiimide to form amide links to the carrier.

Useful carriers are well known in the art, and are generally proteins themselves. Exemplary of such carriers are keyhole limpet hemocyanin (KLH), edestin, thyroglobulin, albumins such as bovine serum albumin (BSA) or human serum albumin (HSA), red blood cells such as sheep erythrocytes (SRBC), tetanus toxoid, cholera toxoid as well as poly amino acids such as poly (D-lysine: D-glutamic acid), and the like.

As is also well known in the art, it is often beneficial to bind a NANBV structural protein to its carrier by means of an intermediate, linking group. As noted above, glutaraldehyde is one such linking group. However, when cysteine is used, the intermediate linking group is preferably an α -maleimidobenxoyl N-hydroxy succinimide (MBS).

Additionally, MBS may be first added to the carrier by an ester-amide interchange reaction. Thereafter, the addition can be followed by addition of a blocked mercapto group such as thiolacetic acid (CH₃COSH) across the maleimido-double bond. After

cleavage of the acyl blocking group, a disulfide bond is formed between the deblocked linking group mercaptan and the mercaptan of the cysteine residue of the protein.

5 Other means of immunopotential include
the use of liposomes and immuno-stimulating complex
(ISCOM) particles. The unique versatility of
liposomes lies in their size adjustability, surface
10 characteristics, lipid composition and ways in which
they can accommodate antigens. Methods to form
liposomes are known in the art. See, for example,
Prescott, Ed., Methods in Cell Biology, Vol. XIV,
Academic Press, NY (1976) p.33 et seq. In ISCOM
15 particles, the cage-like matrix is composed of Quil A,
extracted from the bark of a South American tree. A
strong immune response is evoked by antigenic proteins
or peptides attached by hydrophobic interaction with
the matrix surface.

20 The choice of carrier is more dependent upon
the ultimate use of the immunogen than upon the
determinant portion of the immunogen, and is based
upon criteria not particularly involved in the present
invention. For example, if an inoculum is to be used
in animals, a carrier that does not generate an
25 untoward reaction in the particular animal should be
selected.

4. Administration

30 The vaccines are conventionally administered
parenterally, by injection, for example, either
subcutaneously or intramuscularly. Additional
formulations which are suitable for other modes of
administration include suppositories and, in some
cases, oral formulations. For suppositories,
35 traditional binders and carriers may include, for

example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1-2%. Oral formulations include such
5 normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. The compositions take the form of solutions, suspensions, tablets,
10 pills, capsules, sustained release formulations or powders and contain 10%-95% of active ingredient, preferably 25-70%.

A NANBV structural protein can be formulated into a vaccine as a neutral or salt form.

15 Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the antigen) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic,
20 tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine,
25 trimethylamine, histidine, procaine, and the like.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be immunogenic and effective to induce an immune response. The quantity to be administered
30 to achieve desired full protective immunity depends on the subject to be immunized, capacity of the subject's immune system to synthesize antibodies or induce cell-mediated response, and the degree of protection desired. Precise amounts of active ingredient
35 required to be administered depend on the judgement of

the practitioner and are peculiar to each individual, but generally a dosage suitable for a broad population can be defined. Suitable dosage ranges are of the order of about ten micrograms (ug) to several
5 milligrams (mg), preferably about 10-500 micrograms and more preferably about 100 micrograms active ingredient for each single immunization dose for a human adult. Suitable regimes for initial
10 administration and booster shots are also variable, but are typified by an initial administration followed in two to six week intervals by a subsequent injection or other administration.

A vaccine can also include an adjuvant as part of the excipient. Adjuvants such as complete
15 Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA) for use in laboratory mammals are well known in the art. Pharmaceutically acceptable adjuvants such as alum can also be used. An exemplary vaccine thus comprises one ml of phosphate buffered saline (PBS)
20 containing about 50 to 200 ug NANBV structural protein adsorbed onto about 0.5 mg to about 2.5 mg of alum, or to 0.1% to 1% Al(OH)₃. A preferred vaccine comprises 1 ml of PBS containing 100 ug NANBV structural protein adsorbed onto 2.5 mg of alum carrier.

25 H. Antibody Compositions

An antibody of the present invention is a composition containing antibody molecules that immunoreact with a NANBV structural antigen and with a
30 NANBV structural protein of the present invention (anti-NANBV structural protein antibody molecules). A preferred antibody contains antibody molecules that immunoreact with an epitope present on a polypeptide having an amino acid residue sequence shown in Figure
35 1 from residue 1 to residue 326, preferably that

immunoreacts with a polypeptide having the sequence shown in Figure 1 from residue 1 to residue 74, from residue 49 to residue 120, or from residue 121 to residue 326.

5 In addition, it is preferred that anti-NANBV structural protein antibody molecules do not immunoreact with the C-100-3 antigen described herein, and available in the commercial assay available from Ortho Diagnostics, Inc.

10 An antibody of the present invention is typically produced by immunizing a mammal with an inoculum containing a NANBV structural protein of this invention and thereby induce in the mammal antibody molecules having immunospecificity for the NANBV
15 structural antigens described herein. The antibody molecules are then collected from the mammal and isolated to the extent desired by well known techniques such as, for example, by using DEAE Sephadex to obtain the IgG fraction.

20 To enhance the specificity of the antibody, the antibodies may be purified by immunoaffinity chromatography using solid phase-affixed immunizing NANBV structural protein. The antibody is contacted with the solid phase-affixed NANBV structural protein
25 for a period of time sufficient for the NANBV structural protein to immunoreact with the antibody molecules to form a solid phase-affixed immunocomplex. The bound antibodies are separated from the complex by standard techniques.

30 The antibody so produced can be used, inter alia, in the diagnostic methods and systems of the present invention to detect NANBV structural antigens as described herein present in a body sample.

35 The word "inoculum" in its various grammatical forms is used herein to describe a

composition containing a NANBV structural antigen of this invention as an active ingredient used for the preparation of antibodies immunoreactive with NANBV structural antigens.

5 The preparation and use of an inoculum for production of an antibody of this invention largely parallels the descriptions herein for a vaccine insofar as the vaccine is also designed to induce the production of antibodies and is exemplary of the preparation and use of an inoculum. A key difference
10 is that the inoculum is formulated for use on an animal rather than a human, as is well known.

 A preferred antibody is a monoclonal antibody and can be used in the same manner as
15 disclosed herein for antibodies of the present invention.

 A monoclonal antibody is typically composed of antibodies produced by clones of a single cell called a hybridoma that secretes (produces) but one
20 kind of antibody molecule. The hybridoma cell is formed by fusing an antibody-producing cell and a myeloma or other self-perpetuating cell line. The preparation of such antibodies were first described by Kohler and Milstein, Nature 256:495-497 (1975), which
25 description is incorporated by reference. The hybridoma supernates so prepared can be screened for immunoreactivity with a NANBV structural antigen such as the NANBV structural protein used in the inoculum to induce the antibody-producing cell. Other methods
30 of producing monoclonal antibodies, the hybridoma cell, and hybridoma cell cultures are also well known.

 Also contemplated by this invention is the hybridoma cell, and cultures containing a hybridoma cell that produce a monoclonal antibody of this
35 invention.

It should be understood that in addition to the aforementioned carrier ingredients the pharmaceutical formulation described herein can include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, binders, surface active agents, thickness, lubricants, preservatives (including antioxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient. Typically, a preservative such as merthiolate (1-5000 of a 1% solution) is added to eliminate the risk of microbial contamination, even if sterile techniques were employed in the manufacture of the vaccine.

I. Diagnostic Systems and Methods

1. Diagnostic Systems

A diagnostic system in kit form includes, in an amount sufficient for at least one assay according to the methods described herein, a NANBV structural protein or a fusion protein of the present invention, as a separately packaged reagent. Instructions for use of the packaged reagent are also typically included.

"Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions and the like.

In preferred embodiments, a diagnostic system of the present invention further includes a label or indicating means capable of signaling the formation of a complex containing a recombinant protein.

As used herein, the terms "label" and "indicating means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal to indicate the presence of a complex. Any label or indicating means can be linked to or incorporated in an antibody or monoclonal antibody or used separately, and those atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel proteins, methods and/or systems.

The label can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturing them to form a fluorochrome (dye) that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes such as fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), tetramethylrhodamine isothiocyanate (TRITC), lissamine, rhodamine 8200 sulphonyl chloride (RB 200 SC), a chelate-lanthanide bound (e.g., Eu, Tb, Sm) and the like. A description of immunofluorescence analysis techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody As a Tool, Marchalonis, et al., eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference.

In preferred embodiments, the label is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, alkaline phosphatase or the like. In such cases where the principal label is an enzyme such as

HRP or glucose oxidase, additional reagents are required to visualize the fact that an antibody-antigen complex (immunoreactant) has formed. Such additional reagents for HRP include hydrogen peroxide and an oxidation dye precursor such as diaminobenzidine. An additional reagent useful with HRP is 2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS).

Radioactive elements are also useful labeling agents and are used illustratively herein. An exemplary radiolabeling agent is a radioactive element that produces gamma ray emissions. Elements which themselves emit gamma rays, such as ^{124}I , ^{125}I , ^{128}I , ^{131}I and ^{51}Cr represent one class of gamma ray emission-producing radioactive element indicating groups. Particularly preferred is ^{125}I . Another group of useful labeling means are those elements such as ^{11}C , ^{18}F , ^{15}O and ^{13}N which themselves emit positrons. The positrons so emitted produce gamma rays upon encounters with electrons present in the animal's body. Also useful is a beta emitter, such as ^{111}In , ^3H , ^{35}S , ^{14}C , or ^{32}P .

Additional labels have been described in the art and are suitable for use in the diagnostic systems of this invention. For example, the specific affinity found between pairs of molecules can be used, one as a label affixed to the specific binding agent and the other as a means to detect the presence of the label. Exemplary pairs are biotin:avidin, where biotin is the label; and peroxidase:anti-peroxidase (PAP), where peroxidase is the label.

The linking of labels, i.e., labeling of, polypeptides and proteins is well known in the art. For instance, antibody molecules produced by a hybridoma can be labeled by metabolic incorporation of

radioisotope-containing amino acids provided as a component in the culture medium. See, for example, Galfre et al., Meth. Enzymol., 73:3-46 (1981). The techniques of protein conjugation or coupling through
5 activated functional groups are particularly applicable. See, for example, Aurameas, et al., Scand. J. Immunol., Vol. 8 Suppl. 7:7-23 (1978), Rodwell et al., Biotech., 3:889-894 (1984), and U.S. Pat. No. 4,493,795.

10 The diagnostic systems can also include, preferably as a separate package, a specific binding agent. A "specific binding agent" is a molecular entity capable of selectively binding a reagent species, which in turn is capable of reacting with a
15 product of the present invention but is not itself a protein expression product of the present invention. Exemplary specific binding agents are antibody molecules such as anti-human IgG or anti-human IgM, complement proteins or fragments thereof, protein A,
20 and the like. Preferably the specific binding agent can bind the anti-NANBV antibody to be detected when the antibody is present as part of an immunocomplex.

In preferred embodiments the specific binding agent is labeled. However, when the
25 diagnostic system includes a specific binding agent that is not labeled, the agent is typically used as an amplifying means or reagent. In these embodiments, the labeled specific binding agent is capable of specifically binding the amplifying means when the
30 amplifying means is bound to a reagent species-containing complex.

The diagnostic kits of the present invention can be used in an "ELISA" format to detect the presence or quantity of antibodies in a body fluid
35 sample such as serum, plasma or saliva. "ELISA"

refers to an enzyme-linked immunosorbent assay that employs an antibody or antigen bound to a solid phase and an enzyme-antigen or enzyme-antibody conjugate to detect and quantify the amount of an antigen or antibody present in a sample. A description of the ELISA technique is found in Chapter 22 of the 4th Edition of Basic and Clinical Immunology by D.P. Sites et al., published by Lange Medical Publications of Los Altos, CA in 1982 and in U.S. Patents No. 3,654,090; No. 3,850,752; and No. 4,016,043, which are all incorporated herein by reference.

Thus, in preferred embodiments, the NANBV structural protein or fusion protein of the present invention can be affixed to a solid matrix to form a solid support that is separately packaged in the subject diagnostic systems.

The reagent is typically affixed to the solid matrix by adsorption from an aqueous medium although other modes of affixation, well known to those skilled in the art, can be used.

Useful solid matrices are well known in the art. Such materials include the cross-linked dextran available under the trademark SEPHADEX from Pharmacia Fine Chemicals (Piscataway, NJ); agarose; beads of polystyrene about 1 micron to about 5 millimeters in diameter available from Abbott Laboratories of North Chicago, IL; polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose- or nylon-based webs such as sheets, strips or paddles; or tubes, plates or the wells of a microtiter plate such as those made from polystyrene or polyvinylchloride.

The NANBV structural protein, fusion protein, labeled specific binding agent or amplifying reagent of any diagnostic system described herein can be provided in solution, as a liquid dispersion or as

a substantially dry power, e.g., in lyophilized form. Where the indicating means is an enzyme, the enzyme's substrate can also be provided in a separate package of a system. A solid support such as the before-described microtiter plate and one or more buffers can also be included as separately packaged elements in this diagnostic assay system.

The packages discussed herein in relation to diagnostic systems are those customarily utilized in diagnostic systems. Such packages include glass and plastic (e.g., polyethylene, polypropylene and polycarbonate) bottles, vials, plastic and plastic-foil laminated envelopes and the like.

2. Diagnostic Methods

The present invention contemplates any diagnostic method that results in detecting anti-NANBV structural protein antibodies or NANBV structural antigens in a body fluid sample using a NANBV structural protein, fusion protein or anti-NANBV structural antigen antibody of this invention as an immunochemical reagent to form an immunoreaction product whose amount relates, either directly or indirectly, to the amount of material to be detected in the sample. Those skilled in the art will understand that there are numerous well known clinical diagnostic chemistry procedures in which an immunochemical reagent of this invention can be used to form an immunoreaction product whose amount relates to the amount of specific antibody or antigen present in a body sample.

Various heterogenous and homogenous protocols, either competitive or noncompetitive, can be employed in performing an assay method of this invention. Thus, while exemplary methods are described herein, the invention is not so limited.

To detect the presence of anti-NANBV structural protein antibodies in a patient, a bodily fluid sample such as blood, plasma, serum, urine or saliva from the patient is contacted by admixture under biological assay conditions with a NANBV structural protein, and preferably with a fusion protein of the present invention, to form an immunoreaction admixture. The admixture is then maintained for a period of time sufficient to allow the formation of a NANBV structural protein-antibody molecule immunoreaction product (immunocomplex). The presence, and preferably the amount, of complex can then be detected as described herein. The presence of the complex is indicative of anti-NANBV antibodies in the sample.

In preferred embodiments the presence of the immunoreaction product formed between NANBV structural protein and a patient's antibodies is detected by using a specific binding reagent as discussed herein. For example, the immunoreaction product is first admixed with a labeled specific binding agent to form a labeling admixture. A labeled specific binding agent comprises a specific binding agent and a label as described herein. The labeling admixture is then maintained under conditions compatible with specific binding and for a time period sufficient for any immunoreaction product present to bind with the labeled specific binding agent and form a labeled product. The presence, and preferably amount, of labeled product formed is then detected to indicate the presence or amount of immunoreaction product.

In preferred embodiments the diagnostic methods of the present invention are practiced in a manner whereby the immunocomplex is formed and

detected in a solid phase, as disclosed for the diagnostic systems herein.

Thus, in a preferred diagnostic method, the NANBV structural protein is affixed to a solid matrix to form the solid phase. It is further preferred that the specific binding agent is protein A, or an anti-human Ig, such as IgG or IgM, that can complex with the anti-NANBV structural protein antibodies immunocomplexed in the solid phase with the NANBV structural protein. Most preferred is the use of labeled specific binding agents where the label is a radioactive isotope, an enzyme, biotin or a fluorescence marker such as lanthanide as described for the diagnostic systems, or detailed by references shown below.

In this solid phase embodiment, it is particularly preferred to use a recombinant protein that contains the antigen defined by the amino acid residue sequence shown in Figure 1 from residue 1 to residue 74, as embodied in the fusion protein as described in Example 7.

In another preferred diagnostic method, the NANBV structural protein of the invention is affixed to solid matrix as described above, and dilutions of the biological sample are subjected to the immunocomplexing step by contacting dilutions of sample with the solid surface and removing non-bound materials. Due to the multivalence of antibodies present in biological samples from infected individuals (bivalent for IgG, pentavalent for IgM), subsequent addition of labeled NANBV structural protein of the invention to this admixture will become attached to the solid phase by the sample antibody serving as bridge between the solid phase NANBV structural protein of the invention and the soluble,

labeled NANBV structural protein. The presence of label in the solidphase indicates the presence and preferably the amount of specific antibody in the sample. One skilled in the art can determine a range of dilutions and determine therefrom a concentration of labeled antigen in the solid phase. The biological sample and the labeled NANBV structural protein of the invention can be admixed prior to, or simultaneously with contacting the biological sample with the solid phase allowing the trimolecular complex to form at the solid phase by utilizing the bridging property of bivalent or multivalent specific antibody. As a particularly useful label, biotinylated NANBV structural protein of the invention can be the labeled antigen, allowing the subsequent detection by addition of an enzyme-streptavidin, or an enzyme-avidin complex, followed by the appropriate substrate. Enzymes such as horse-radish peroxidase, alkaline phosphatase, β -galactosidase or urease are frequently used and these, and other, along with several appropriate substrates are commercially available. Preferred labels with a marker which allows direct detection of the formed complex include the use of a radioactive isotope, such as, eg., iodine, or a lanthanide chelate such as Europium.

In another embodiment designed to detect the presence of a NANBV structural antigen in a body fluid sample from a patient, the sample (e.g. blood, plasma, serum, urine or saliva) is contacted by admixture under biological assay conditions with an anti-NANBV structural protein antibody of this invention, to form an immunoreaction admixture. The admixture is then maintained for a period of time sufficient to allow the formation of a antigen-antibody immunoreaction product containing NANBV structural antigens complexed

with an antibody of this invention. The presence and preferably amount, of complex can then be determined, thereby indicating the presence of antigen in the body fluid sample.

5 In a preferred embodiment, the antibody is present in a solid phase. Still further preferred, the amount of immunocomplex formed is measured by a competition immunoassay format where the antigen in a patient's body fluid sample competes with a labeled
10 recombinant antigen of this invention for binding to the solid phase antibody. The method comprises admixing a body fluid sample with (1) solid support having affixed thereto an antibody according to this invention and (2) a labeled NANBV structural protein
15 of this invention to form a competition immunoreaction admixture that has both a liquid phase and a solid phase. The admixture is then maintained for a time period sufficient to form a labeled NANBV structural protein-containing immunoreaction product in the solid
20 phase. Thereafter, the amount of label present in the solid phase is determined, thereby indicating the amount of NANBV structural antigen in the body fluid sample.

25 Enzyme immunoassay techniques, whether direct or competition assays using homogenous or heterogenous assay formats, have been extensively described in the art. Exemplary techniques can be found in Maggio, Enzyme Immunoassay, CRC Press, Cleveland, OH (1981); and Tijssen, "Practice and
30 Theory of Enzyme Immunoassays", Elsevier, Amsterdam (1988).

35 Biological assay conditions are those that maintain the biological activity of the NANBV structural protein and the anti-NANBV structural protein antibodies in the immunoreaction admixture.

Those conditions include a temperature range of about 4 C to about 45 C, preferably about 37 C, a pH value range of about 5 to about 9, preferably about 7, and an ionic strength varying from that of distilled water to that of about one molar sodium chloride, preferably about that of physiological saline. Methods for optimizing such conditions are well known in the art.

Also contemplated are immunological assays capable of detecting the presence of immunoreaction product formation without the use of a label. Such methods employ a "detection means", which means are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel polypeptides, methods and systems. Exemplary detection means include methods known as biosensors and include biosensing methods based on detecting changes in the reflectivity of a surface (surface plasmon resonance), changes in the absorption of an evanescent wave by optical fibers or changes in the propagation of surface acoustical waves.

Another embodiment contemplates detection of the immunoreaction product employing time resolved fluorometry (TR-FIA), where the label used is able to produce a signal detectable by TR-FIA. Typical labels suitable for TR-FIA are metal-complexing agents such as a lanthanide chelate formed by a lanthanide and an aromatic beta-diketone, the lanthanide being bound to the antigen or antibody via an EDTA-analog so that a fluorescent lanthanide complex is formed.

The principle of time-resolved fluorescence is described by Soini et al, Clin. Chem., 25:353-361 (1979), and has been extensively applied to immunoassay. See for example, Halonen et al., Current Topics in Microbiology and Immunology, 104: 133-146

(1985); Suonpaa et al., Clinica Chimica Acta, 145:341-348 (1985); Lovgren et al., Talanta, 31:909-916 (1984); U.S. Patent Nos. 4,374,120 and 4,569,790; and published International Patent Application Nos. EPO 139 675 and W087/02708. A preferred lanthanide for use in TR-FIA is Europium.

Regents and systems for practicing the TR-FIA technology are available through commercial suppliers (Pharmacia Diagnostics, Upsala, Sweden).

Particularly preferred are the solid phase immunoassays described herein in Example 7, performed as a typical "Western Blot".

The present diagnostic methods may be practiced in combination with other separate methods for detecting the appearance of anti-NANBV antibodies in species infected with NANBV. For example, a composition of this invention may be used together with commercially available C-100-3 antigen (Ortho Diagnostics, Inc., Raritan, N.J.) in assays to determine the presence of either or both antibody species immunoreactive with the two antigens.

Examples

The following examples are given for illustrative purposes only and do not in any way limit the scope of the invention.

1. Production of Recombinant DNA Molecules

A. Isolation of NANBV Clones and Sequence Analysis

(1) Isolation of NANBV RNA and Preparation of cDNA

As a source for NANB virions, blood was collected from a chimpanzee infected with the Hutchinson (Hutch) strain exhibiting acute phase NANBH. Plasma was clarified by centrifugation and

filtration. NANB virions were then isolated from the clarified plasma by immunoaffinity chromatography on a column of NANBV IgG (Hutch strain) coupled to protein G sepharose. NANBV RNA was eluted from the sepharose beads by soaking in guanidinium thiocyanate and the eluted RNA was then concentrated through a cesium chloride (CsCl) cushion. Maniatis et al., Molecular Cloning: A Laboratory Manual, Maniatis et al., eds. Cold Spring Harbor, New York (1989).

The purified NANBV RNA was used as a template in a primer extension reaction admixture containing random and oligo dT primers, dNTP's, and reverse transcriptase to form first strand cDNAs. The resultant first strand cDNAs were used as templates for synthesis of second strand cDNAs in a reaction admixture containing DNA polymerase I and RNase H to form double stranded (ds) cDNAs (Maniatis et al., Supra). The synthesized ds cDNAs were amplified using an assymetric synthetic primer-adaptor system wherein sense and anti-sense primers were annealed to each other and ligated to the ends of the double stranded NANBV cDNAs with T4 ligase under blunt-end conditions to form cDNA-adaptor molecules. Polymerase chain reaction (PCR) amplification was performed by admixing the cDNA-adaptor molecules with the same positive sense adaptor primers, dNTP's and TAQ polymerase to prepare amplified NANBV cDNAs. The resultant amplified NANBV cDNA sequences were then used as templates for subsequent amplification in a PCR reaction with specific NANBV oligonucleotide primers.

(2) Synthesis of Oligonucleotides
For Use in NANBV Cloning

Oligonucleotides were selected to correspond to the 5' sequence of Hepatitis C which putatively encodes the NANBV structural capsid and

envelope proteins (HCV1 sequence: Okamoto et al.,
Jap. J. Exp. Med., 60:167-177, 1990). The selected
oligonucleotides were synthesized on a Pharmacia Gene
Assembler according to the manufacturer's instruction,
5 purified by polyacrylamide gel electrophoresis and
have nucleotide base sequences as shown in Table 1.

TABLE 1
Synthetic Oligonucleotides

Oligo- nucleotide	Putative NANBV	Oligonucleotide
<u>Designation*</u>	<u>Region</u>	<u>Sequence</u>
690 (+)	Capsid 1-21	ATGAGCACGATTCCCAAACCT
693 (+)	Capsid 146-162	GAGGAAGACTTCCGAGC
694 (-)	Capsid 208-224	GTCCTGCCCTCGGGCCG
691 (-)	Capsid 340-360	ACCCAAATTGCGCGACCTACG
14 (+)	Envelope 356-374	TGGGTAAGGTCATCGATAC
15 (+)	Envelope 361-377	AAGGTCATCGATACCCT
18 (-)	Envelope 512-529	AGATAGAGAAAGAGCAAC
16 (-)	Envelope 960-981	GGACCAAGTTCATCATATATAT
17 (-)	Envelope 957-976	CAGTTCATCATCATATCCCA

* The oligonucleotides are numerically defined and
their polarity is indicated as (+) and (-) for sense
and anti-sense, respectively.

(3) PCR Amplification of NANBV
cDNA

PCR amplification was performed by
admixing the primer-adapted amplified cDNA sequences
prepared in Example 1.A.(1) with the synthetic
35 oligonucleotides 690 and 694 as primer (primer pairs

690:694). The resulting PCR reaction admixture contained the primer-adapted amplified cDNA template, oligonucleotides 690 and 694, dNTP's, salts (KCl and MgCl₂) and TAQ polymerase. PCR amplification of the cDNA was conducted by maintaining the admixture at a 37 C annealing temperature for 30 cycles. Aliquots of samples from the first round of amplification were reamplified at a 55 C annealing temperature for 30 cycles under similar conditions.

(4) Preparation of Vectors
Containing PCR Amplified ds
DNA

Aliquots from the second round of PCR amplification were subjected to electrophoresis on a 5% acrylamide gel. After separation of the PCR reaction products, the region of the gel containing DNA fragments corresponding to the expected 690:694 amplified product of approximately 224 bp was excised and purified following standard electroelution techniques (Maniatis et al., Supra). The purified fragments were kinased and cloned into the pUC 18 plasmid cloning vector at the Sma I polylinker site to form a plasmid containing the DNA segment 690:694 operatively linked to pUC 18.

The resulting mixture containing pUC 18 and a DNA segment corresponding to the 690:694 sequence region was then transformed into the E. coli strain JM83. Plasmids containing inserts were identified as lac⁻ (white) colonies on Xgal medium containing ampicillin. pUC 18 plasmids which contained the 690:694 DNA segment were identified by restriction enzyme analysis and subsequent electrophoresis on agarose gels, and were designated pUC 18 690:694 rDNA molecules.

(5) Sequencing of Hepatitis
Clones that Encode the
Putative Capsid Protein

Two independent colonies believed
5 to contain a pUC 18 vector having the NANBV Hutch
strain 690:694 DNA segment (pUC 18 690:694) that codes
for the amino terminus of the putative capsid protein
were amplified and used to prepare plasmid DNA by
CsCl density gradient centrifugation by standard
10 procedures (Maniatis et al., Supra). The plasmids
were sequenced using ³⁵S dideoxy procedures with pUC
18 specific primers. The two plasmids were
independently sequenced on both DNA strands to assure
the accuracy of the sequence. The resulting sequence
15 information is presented as nucleotides 1-224 of
Figure 1.

Plasmid pUC 18 690:694 contains a NANBV DNA
segment that is 224 bp in length and when compared to
the HCV1 prototype sequence reveals two nucleotide
20 substitutions and one amino acid residue difference in
the amino terminal region of the putative capsid
protein.

(6) Preparation of NANBV Clones
from the 5' End of the Genome

25 To obtain the sequence of the
NANBV Hutch genome encoding the remainder of the
capsid region (Okamoto et al., Supra), the
oligonucleotides 693 and 691 (described in Table 1)
were used in PCR reactions. cDNA was prepared as
described in Example 1.A.(1) to viral NANBV RNA from
30 (Hutch) and used in PCR amplification as described in
Example 1.A.(3) with the oligonucleotide pair 693:691.
The resultant PCR amplified ds DNA was then cloned
into pUC 18 cloning vectors and screened for inserts
35 as described in Example 1.A.(4) to form pUC 18

693:691. Clones were then sequenced with pUC 18 specific primers as described in Example 1.A.(5).

Plasmid pUC 18 693:691 contains a NANBV DNA segment that is 157 bp in length and spans nucleotides 203-360 (Figure 1). The clone is not complete to the 693 primer used for generating the fragment. The sequence of this fragment reveals three nucleotide differences when compared to the known sequence of HCJ1 and does not have any corresponding amino acid changes to the HCJ1 sequence.

To obtain the sequence of the NANBV Hutch genome encoding the putative envelope region (Okamoto et al., Supra), the oligonucleotide primers 14 through 18 (described in Table 1) were used in various combinations with NANBV Hutch RNA samples. As a source of NANBV RNA, a liver biopsy specimen from a chimpanzee inoculated with the Hutch strain at 4 weeks post-inoculation and exhibiting acute infection was used. The biopsied sample was first frozen and then ground. The resultant powder was then subjected to treatment with guanidine isothiocyanate for the extraction of RNA. RNA was extracted from the guanidium treated liver samples with phenol in the presence of SDS at 65 C. The liver samples were extracted a second time, and subjected to extraction with chloroform. The extracted RNA was precipitated at -20 C with isopropanol and sodium acetate.

The purified liver-derived RNA was used as a template in primer extension reactions with the oligonucleotides 18 and 16 to generate NANBV specific cDNAs. To prepare cDNA to the Hutch strain amino-terminal protein coding sequences, anti-sense oligonucleotides, 18 and 16, were annealed to liver-derived Hutch RNA in the presence of dNTPs and reverse transcriptase at 42 C to form primer extension

products. The first round of PCR amplification of the two cDNAs was performed by admixing the primer extension reaction products with separate pairs of oligonucleotides 14:16 (16 primed cDNA) and 14:18 (18 primed cDNA) for 30 cycles at 55 C annealing temperature. The PCR reactions were performed on the above admixture as in 1.A.(3). Aliquots from the 14:16 and 14:18 amplifications were used as templates for the second round of amplification in which the oligonucleotide pairs 15:17 and 15:18, respectively, were used as primers.

PCR reaction products from each of the primer pair reactions were analyzed by electrophoresis on low melt agarose gels. Following separation, the regions of the gel containing DNA fragments corresponding to the expected 15:17 and 15:18 amplified products of approximately 617 bp and 168 bp, respectively, were excised and eluted from the gel slices at 65 C. The resultant eluted fragments were purified by phenol and chloroform extractions. To clone the 15:17 and 15:18 fragments, the purified fragments were separately treated with the Klenow fragment of DNA polymerase and kinase for subsequent subcloning into the Sma I site of the pBluescript plasmid vector (Stratagene Cloning Systems, La Jolla, CA). Transformed *E. coli* DH5 colonies were analyzed for plasmid insert by restriction enzyme analysis as described in Example 1.A.(4).

pBluescript plasmid containing 15:17 or 15:18 DNA segments were purified using large scale CsCl plasmid preparation protocols. The DNA segments present in the amplified and purified plasmids were each sequenced as described in Example 1.A.(5).

The sequence of the 15:17 DNA segment is shown in Figure 1 from nucleotide 361 to 978. The

sequence of the 15:18 DNA segment is also presented in Figure 1 from nucleotide 361 to 529. These two clones overlap by 168 bp of the 15:18 DNA segment.

The sequence results indicate that the 15:17 DNA segment differs by 30 nucleotides when compared to the HCJ1 sequence (Okamoto et al., Supra) and also differs by ten amino acid residues. The 15:18 DNA segment differs by seven nucleotides and by three amino acid residues when compared to HCJ1. In the overlap region, the two DNA segments differ at two nucleotide bases, namely, bases 510 and 511, where DNA segment 15:18 contains a T in place of a C and a G in place of an A, respectively, which results in a change of a serine in place of a glycine amino acid residue, at residue 171 of Figure 1. The reason for these differences is unknown and may be due to a PCR artifact.

B. Production of Recombinant DNA
(rdNA) that Encodes a Fusion
Protein

(1). Isolation of the 690:694
Fragment from the pUC 18
Clone and Introduction of the
Fragment into the pGEX-3X
Expression Vector

The pUC 18 vector containing the 690:694 DNA segment was subjected to restriction enzyme digestion with Eco RI and Bam HI to release the DNA segment having a sequence shown in Figure 1 from base 1 to base 224 from the pUC 18 vector. The released DNA segment was subjected to acrylamide electrophoresis and a DNA segment containing the 224 bp NANBV insert plus portions of the pUC 18 polylinker was then excised and eluted from the gel as described in Example 1.A.(4). The DNA segment was extracted

with a mixture of phenol and chloroform, and precipitated.

The precipitated DNA segment was resuspended to a concentration of 25 ug/ml in water and treated with the Klenow fragment of DNA polymerase to fill in the staggered ends created by the restriction digestion. The resultant blunt-ended 690:694 segment was admixed with the bacterial expression vector, pGEX-3X, (Pharmacia Inc., Piscataway, N.J.) which was linearized with the blunt end restriction enzyme Sma I. The admixed DNAs were then ligated by maintaining the admixture overnight at 16 C in the presence of ligase buffer and 5 units of T4 DNA ligase to form a plasmid of 690:694 DNA segment operatively linked to pGEX-3X.

(2). Selection and Verification of
Correct Orientation of
Ligated Insert

The ligation mixture containing the pGEX-3X vector and the 690:694 DNA segment was transformed into host E. coli strain W3110. Plasmids containing inserts were identified by selection of host bacteria containing vector in Luria broth (LB) media containing ampicillin. Bacterial cultures at stationary phase were subjected to alkaline lysis protocols to form a crude DNA preparation. The DNA was digested with the restriction enzyme Xho I. The single Xho I site, which cleaves within the 690:694 DNA segment between nucleotide position 173-178 (Figure 1), but not within the pGEX-3X vector, was used to screen for vector containing the 690:694 DNA segment.

Several 690:694 DNA segment-containing vectors were amplified and the resultant amplified vector DNA was purified by CsCl density gradient

centrifugation. The DNA was sequenced across the inserted DNA segment ligation junctions by ³⁵S dideoxy methods with a primer which hybridized to the pGEX-3X sequence at nucleotide positions 614 to 633 shown in Figure 2. Vectors containing 690:694 DNA segment having the correct coding sequence for in-frame translation of a NANBV structural protein were thus identified and selected to form pGEX-3X-690:694.

(3). Structure of the Fusion

Protein

The pGEX-3X vector is constructed to allow for inserts to be placed at the C terminus of Sj26, a 26-kDa glutathione S-transferase (GST; EC 2.5.1.18) encoded by the parasitic helminth Schistosoma japonicum. The insertion of the 690:694 NANBV fragment in-frame behind Sj26 allows for the synthesis of the Sj26-NANBV fusion polypeptide. The NANBV polypeptide can be cleaved from the GST carrier by digestion with the site-specific protease factor Xa (Smith et al., Gene, 67:31-40, 1988).

The nucleotide and predicted amino acid sequence of the pGEX-3X-690:694 fusion transcript from the GST sequence through the 690:694 insert is presented in Figure 2. The resulting rDNA molecule, pGEX-3X-690:694, is predicted to encode a NANBV fusion protein having the amino acid residue sequence shown in Figure 2 from amino acid residue 1 to residue 315. The resulting protein product generated from the expression of the plasmid is referred to as the NANBV capsid protein amino terminus (CAP-N).

C. Production of Recombinant DNAs
(rDNAs) that Encode NANBV Capsid
and Envelope Fusion Proteins

pGEX-3X-693:691: Plasmid

pGEX-3X-693:691 was formed by first subjecting the

plasmid pUC 18 693:691 prepared in Example 1.A.(6) to restriction enzyme digestion with Eco RI and Bam HI as performed in Example 1.B.(1). The resultant released DNA segment having a sequence shown in Figure 1 from base 205 to base 360 was purified as performed in Example 1.B.(1). The purified DNA segment was admixed with and ligated to the pGEX-3X vector which was linearized by restriction enzyme digestion with Eco RI and Bam HI in the presence of T₄ ligase at 16 C to form the plasmid pGEX-3X-693:691.

A pGEX-3X plasmid containing a 693:691 DNA segment was identified by selection Example 1.B.(2) with the exception that crude DNA preparations were digested with Eco RI and Bam HI to release the 693:691 insert. A pGEX-3X vector containing a 693:691 DNA segment having the correct coding sequence for in-frame translation of a NANBV structural protein was identified by sequence analysis as performed in Example 1.B.(2) and selected to form pGEX-3X-693:691.

The resulting vector encodes a fusion protein (GST:NANBV 693:691) that is comprised of an amino-terminal polypeptide portion corresponding to residues 1-221 of GST as shown in Figure 2, an intermediate polypeptide portion corresponding to residues 222-225 and defining a cleavage site for the protease Factor Xa, a linker protein corresponding to residues 226-230 consisting of the amino acid residue sequence:

Gly Ile Pro Asn Ser

encoded by the nucleotide base sequence:

GGG ATC CCC AAT TCA, respectively;

a carboxy-terminal polypeptide portion corresponding to residues 231-282 defining a NANBV capsid antigen as shown by the amino acid residue sequence 69-120 in

Figure 1, and a carboxy-terminal linker portion corresponding to residues 283-287 consisting of the amino acid residue sequence:

Asn Ser Ser END.

5 encoded by the nucleotide base sequence:

AAT TCA TCG TGA, respectively.

pGEX-3X-15:18: Plasmid pGEX-3X-15:18 was formed by first subjecting the plasmid Bluescript 15:18 prepared in Example 1.A.(6) to restriction enzyme digestion with Eco RV and Bam HI and the Bam HI cohesive termini were filled in as performed in 10 Example 1.B.(1). The resultant released DNA segment having a sequence shown in Figure 1 from base 361 to base 528 was purified as performed in Example 1.B.(1). 15 The purified DNA segment was admixed with and ligated to the pGEX-3X vector which was linearized by restriction enzyme digestion with Sma I as performed in 1.B.(1) to form the plasmid pGEX-3X-15:18.

A pGEX-3X plasmid containing a 15:18 DNA segment was identified by selection as performed 20 in Example 1.B.(2) and crude DNA preparations were cut with Eco RI and Bam HI to release the 15:18 inserts. A pGEX-3X vector containing a 15:18 DNA segment having the correct coding sequence for in-frame translation 25 of a NANBV structural protein was identified as performed in Example 1.B.(2) and selected to form pGEX-3X-15:18.

The resulting vector encodes a fusion protein (GST:NANBV 15:18) that is comprised of an amino-terminal polypeptide portion corresponding to 30 residues 1-221 of GST; an intermediate polypeptide portion corresponding to residues 222-225 and defining a cleavage site for the protease Factor Xa, a linker protein corresponding to residues 226-234 consisting 35 of the amino acid residue sequence:

Gly Ile Pro Ile Glu Phe Leu Gln Pro,
encoded by the nucleotide base sequence:

GGG ATC CCC ATC GAA TTC CTG CAG CCC,
respectively; a carboxy-terminal polypeptide portion
5 corresponding to residues 235-290 defining a NANBV
envelope antigen as shown by the amino acid residue
sequence 121-176 in Figure 1, and a carboxy-terminal
linker portion corresponding to residues 291-298
consisting of a amino acid residue sequence:

10 Trp Gly Ile Gly Asn Ser Ser END
encoded by the nucleotide base sequence:

TGG GGG ATC GGG AAT TCA TCG TGA,
respectively.

pGEX-3X-15:17: Plasmid pGEX-3X-15:17
15 was formed by first subjecting the plasmid Bluescript
15:17 prepared in Example 1.A.(6) to restriction
enzyme digestion with Eco RI and Bam HI and the
cohesive termini were filled in as performed in
Example 1.B.(1). The resultant released DNA segment
20 having a sequence shown in Figure 1 from base 361 to
base 978 was purified as performed in Example 1.B.(1).
The purified DNA segment was admixed with and ligated
to the pGEX-3X vector which was linearized by
restriction enzyme digestion with Sma I as performed
25 in Example 1.B.(1) to form the plasmid pGEX-3X-15:17.

A pGEX-3X plasmid containing a 15:17
DNA segment was identified by selection as performed
in Example 1.B.(2) and DNA preparations were digested
with Eco RI and Bam HI as indicated above. pGEX-3X
30 vector containing a 15:17 DNA segment having the
correct coding sequence for in-frame translation of a
NANBV structural protein was identified as performed
in Example 1.B.(2) and selected to form pGEX-3X-15:17.

The resulting vector encodes a fusion protein (GST:NANBV 15:17) that is comprised of an amino-terminal polypeptide portion corresponding to residues 1-221 of GST, an intermediate polypeptide portion corresponding to residues 222-225 and defining a cleavage site for the protease Factor Xa, a linker protein corresponding to residues 226-233 consisting of the amino acid residue sequence:

Gly Ile Pro Asn Leu Arg Ser Pro

encoded by the nucleotide base sequence:

GGG ATC CCC AAT TCC TGC AGC CCT,

respectively; a carboxy-terminal polypeptide portion corresponding to residues 234-439 defining a NANBV envelope antigen as shown by the amino acid residue sequence 121-326 in Figure 1, and a carboxy-terminal linker portion corresponding to residues 440-446 consisting of the amino acid residue sequence:

Gly Ile Gly Asn Ser Ser END

encoded by the nucleotide base sequence:

GGG ATC GGG AAT TCA TCG TGA, respectively.

pGEX-2T-15:17: Plasmid pGEX-2T-15:17 was formed by first subjecting the plasmid Bluescript 15:17 prepared in Example 1.A.(6) to restriction enzyme digestion with Eco RV and Bam HI and the Bam HI cohesive termini were filled in as performed in Example 1.B.(1). The resultant released DNA segment having a sequence shown in Figure 1 from base 361 to base 978 was purified as performed in Example 1.B.(1). The purified DNA segment was admixed with and ligated to the pGEX-2T vector (Pharmacia, INC.) which was linearized by restriction enzyme digestion with Sma I as performed in Example 1.B.(1) to form the plasmid pGEX-2T-15:17.

A pGEX-2T plasmid containing a 15:17 DNA segment was identified by selection as performed

in Example 1.B.(2) and by digestion of crude DNA preparations with Eco RI and Bam HI. A pGEX-2T vector containing a 15:17 DNA segment having the correct coding sequence for in-frame translation of a NANBV structural protein was identified as performed in
5 Example 1.B.(2) and selected to form pGEX-2T-15:17.

The resulting vector encodes a fusion protein (GST:NANBV 15:17) that is comprised of an amino-terminal polypeptide portion corresponding to
10 residues 1-221 of GST, an intermediate polypeptide portion corresponding to residues 222-226 and defining a cleavage site for the protease Thrombin consisting of the amino acid residue sequence:

Val Pro Arg Gly Ser

15 encoded by the nucleotide base sequence:

GTT CCG CGT GGA TCC, respectively;

a linker protein corresponding to residues 227-233 consisting of an amino acid residue sequence:

Pro Ser Asn Leu Arg Ser Pro

20 encoded by a nucleotide base sequence:

CCA TCG AAT TCC TGC AGC CCT,

respectively; a carboxy-terminal polypeptide portion corresponding to residues 234-439 defining a NANBV envelope antigen, and a carboxy-terminal linker
25 portion corresponding to residues 440-446 consisting of the amino acid residue sequence:

Gly Ile His Arg Asp END

encoded by the nucleotide base sequence

GGA ATT CAT CGT GAC TGA, respectively.

30 pGEX-3X-690:691: To obtain a DNA segment corresponding to the NANBV Hutch sequence sequence shown from Figure 1 from base 1 to base 360, the oligonucleotides 690:691 are used in PCR reactions as performed in Example 1.A.(6). The resultant PCR
35 amplified ds DNA is then cloned into pUC 18 cloning

vectors as described in Example 1.A.(4) to form pUC 18 690:691. Clones are then sequenced with pUC 18 primers as described in Example 1.A.(5) to identify a plasmid containing the complete sequence. The
5 resulting identified plasmid is selected, is designated pUC 18 690:691, and contains a NANBV DNA segment that is 360 bp in length and spans nucleotides 1-360 (Figure 1).

10 Plasmid pGEX-3X-690:691 is formed by first subjecting the plasmid pUC 18 690:691 to restriction enzyme digestion with Eco RI and Bam HI as performed in Example 1.B.(1). The resultant released DNA segment having a sequence shown in Figure 1 from base 1 to base 360 with pUC 18 polylinker sequence is
15 purified as performed in Example 1.B.(1). The purified DNA segment is admixed with and ligated to the pGEX-3X vector which is linearized by restriction enzyme digestion with Sma I as performed in Example 1.B.(1) to form the plasmid pGEX-3X-690:691.

20 A pGEX-3X plasmid containing a 690:691 DNA segment is identified by selection as performed in Example 1.B.(2). pGEX-3X vector containing a 690:691 DNA segment having the correct coding sequence for in-frame translation of a NANBV structural protein is
25 identified as performed in Example 1.B.(2) and selected to form pGEX-3X-690:691.

B
30 The resulting vector encodes a fusion protein (GST:NANBV 690:691) that is comprised of an ~~amino~~ ^{amino}-terminal polypeptide portion corresponding to residues 1-221 of GST, an intermediate polypeptide portion corresponding to residues 222-225 and defining a cleavage site for the protease Factor Xa, a linker protein corresponding to residues 226-234 consisting of the amino acid residue sequence:

35 Gly Ile Pro Asn Ser Ser Ser Val Pro

encoded by the nucleotide base sequence:

GGG ATC CCC AAT TCG AGC TCG GTA CCC

respectively; a carboxy-terminal polypeptide portion
corresponding to residues 235-355 defining a NANBV
capsid antigen, and a carboxy-terminal linker portion
corresponding to residues 356-363 consisting of the
amino acid residue sequence:

Thr Gly Ile Gly Asn Ser Ser END

encoded by the nucleotide base sequence:

ACG GGG ATC GGG AAT TCA TCG TGA,

respectively.

2. Expression of the NANBV 690:694 Fusion
Protein Using rDNA

The bacterial colonies which contain the
pGEX-3X-690:694 plasmid in the correct orientation
were selected to examine the properties of the fusion
protein. Bacterial cultures of pGEX-3X-690:694 were
grown to a stationary phase in the presence of
ampicillin (50 ug/ml final concentration) at 37 C.

This culture was inoculated at a 1:50 dilution into
fresh LB medium at 37 C in the presence of ampicillin
and maintained at 37 C. with agitation at 250 rpm
until the bacteria reached an optical density of 0.5
when measured using a spectrometer with a 550 nm
wavelength light source detector. Isopropylthio-
beta-D-galactoside (IPTG) was then admixed to the
bacterial culture at a final concentration of 1 mM to
initiate (induce) the synthesis of the fusion proteins
under the control of the tac promoter in the pGEX-3X
vector.

Beginning at zero time and at one hour
intervals thereafter for three hours following
admixture with IPTG (i.e., the induction phase), the
bacterial culture was maintained as above to allow
expression of recombinant protein. During this

5 maintenance phase, the optical density of the
bacterial culture was measured and 1 ml aliquots were
removed for centrifugation. Each resultant cell
pellet containing crude protein lysate was resuspended
in Laemmli dye mix containing 1% beta-mercaptoethanol
at a final volume of 50 microliters (ul) for each 0.5
OD 550 unit. Samples were boiled for 15 minutes and
10 ul of each sample was electrophoresed on a 10% SDS-
PAGE Laemmli gel.

10 3. Detection of Expressed Fusion Proteins

To visualize the IPTG-induced fusion
proteins, the Laemmli gels were stained with Coomassie
Blue and destained in acetic acid and methanol.
Induced proteins from separate clones were examined
and compared on the basis of the increase of a protein
band in the predicted size range from time zero to
time three hours post-IPTG treatment. Expression of
fusion protein was observed in clones that exhibited
an increase from zero time of the intensity of a
protein band corresponding to the fusion protein.

20 4. Western Blot Analysis

Samples from IPTG inductions were separated
by gel electrophoresis and were transferred onto
nitrocellulose for subsequent immunoblotting analysis.
The nitrocellulose filter was admixed with antibody
blocking buffer (20 mM sodium phosphate, pH 7.5, 0.5 M
sodium chloride, 1% bovine serum albumin, and 0.05%
Tween 40) for 3 to 12 hours at room temperature. Sera
from humans or chimpanzees with NANB hepatitis
believed to contain antibody immunoreactive with NANBV-
structural protein was diluted 1:500 in the antibody
blocking buffer and admixed with the nitrocellulose
and maintained for 12 hours at room temperature to
allow the formation of an immunoreaction product on
the solid phase. The nitrocellulose was then washed

three times in excess volumes of antibody blocking buffer. The washes were followed by admixture of the nitrocellulose with 50 μ l of 125 I protein A (New England Nuclear, Boston, MA) at a 1:500 dilution in antibody blocking buffer for one hour at room temperature to allow the labeled protein A to bind to any immunoreaction product present in the solid phase on the nitrocellulose. The nitrocellulose was then washed as described herein, dried and exposed to X-ray film for one to three hours at -70 C in order to visualize the label and therefore any immunoreaction product on the nitrocellulose. Results of the Western blot immunoassay are shown in Tables 2 through 6. Samples prepared using pGEX-3X vector that produces control GST were also prepared as above and tested using the Western blot procedure as a control. No expressed protein (GST) was detectable having immunoreactivity with the sera shown to immunoreact with a fusion protein of this invention (GST:NANBV 690:694 fusion protein).

5. Purification of the Expressed GST:NANBV 690:694 Fusion Protein

Cultures of E. coli strain W3110 transformed with recombinant pGEX-3X-690:694 plasmids prepared in Example 2 were cultured for 3 hours following IPTG induction treatment. The cells were then centrifuged to form a bacterial cell pellet, the cells were resuspended in 1/200 culture volume in lysis buffer (MTPBS: 150 mM NaCl, 16 mM Na_2HPO_4 , 4 mM NaH_2PO_4 , pH 7.3), and the cell suspension was lysed with a French pressure cell. Triton X-100 was admixed to the cell lysate to produce a final concentration of 1%. The admixture was centrifuged at 50,000 X g for 30 minutes at 4 C. The resultant supernatant was collected and admixed with 2 ml of 50% (w/v) glutathione agarose

beads (Sigma, St. Louis, MO) preswollen in MTPBS. After maintaining the admixture for 5 minutes at 25 degrees C to allow specific affinity binding between GST and glutathione in the solid phase, the beads were collected by centrifugation at 1000 X g and washed in MTPBS three times.

The GST:NANBV 690:694 fusion protein was eluted from the washed glutathione beads by admixture and incubation of the glutathione beads with 2 ml of 50 mM Tris HCl, pH 8.0, containing 5 mM reduced glutathione for 2 minutes at 25 degrees C to form purified GST:NANBV 690:694 fusion protein.

The above affinity purification procedure produced greater than 95% pure fusion protein as determined by SDS PAGE. That is, the purified protein was essentially free of procaryotic antigen and non-structural NANBV antigens as defined herein.

Alternatively, GST:NANBV 690:694 fusion protein was purified by anion exchange chromatography. Cultures were prepared as described above and cell pellets were resuspended in 8M guanidine and maintained overnight at 4 C to solubilize the fusion protein. The cell suspension was then applied to an S-300 sepharose chromatography column and peak fractions containing the GST:NANBV 690:694 fusion protein were collected, pooled, dialyzed in 4 M urea and subjected to anion exchange chromatography to form purified fusion protein.

6. Protease Cleavage of Purified GST:NANBV 690:694 Fusion Protein

Purified GST:NANBV 690:694 fusion protein prepared in Example 5 is subjected to treatment with activated Factor (Xa) (Sigma) to cleave the GST carrier from the NANBV 690:694 fusion protein (Smith et al., Supra). Seven ug of Factor X are activated

prior to admixture with purified fusion proteins by admixture and maintenance with 75 nanograms (ng) activation enzyme, 8 mM Tris HCl (pH 8.0), 70 mM NaCl and 8 mM CaCl₂ at 37 C for 5 minutes. Fifty ug of purified fusion protein are then admixed with 500 ng activated human factor Xa in the elution buffer described in Example 5 containing 50 mM Tris HCl, 5 mM reduced glutathione, 100 mM NaCl, and 1 mM CaCl₂, and maintained at 25 C for 30 minutes. The resulting cleavage reaction products are then absorbed on glutathione-agarose beads prepared in Example 5 to affinity bind and separate free GST from any cleaved NANBV structural antigen-containing protein. Thereafter the liquid phase is collected to form a solution containing purified NANBV structural protein having an amino acid residue sequence shown in Figure 2 from residue 226 to residue 315.

7. Immunological Detection of Anti-NANBV Structural Protein Antibodies

NANBV Hutch strain virus was injected in chimpanzees and blood samples were collected at various intervals to analyze the immunological response to NANBV by five different diagnostic assays. Chimpanzees were categorized as either being in the acute or chronic phase of infection. The assays utilized in the evaluation of the immune response include: 1) Alanine aminotransferase (ALT) enzyme detection (Alter et al., JAMA, 246:630-634, 1981; Aach et al., N. Engl. J. Med., 304:989-994, 1981); 2) Histological evaluation for NANBV virions by electron microscopy (EM); 3) Detection of anti-HCV antibodies using the commercially available kit containing C-100-3 antigen (Ortho Diagnostics, Inc.); 4) Detection of anti-CAP-N antibodies by immunoblot analysis as

described in Example 4; and 5) Detection of virus by PCR amplification as described in Example 1.

In Table 2, results are presented from ALT, EM, anti-HCV, anti-CAP-N, and PCR assays on sera from a chimpanzee with acute NANB Hepatitis.

TABLE 2

CHIMP 59 - ACUTE NANB HEPATITIS

WEEK					PCR
POST			ANTI	ANTI	690-
<u>INNOV</u>	<u>ALT</u>	<u>EM</u>	<u>HCV</u>	<u>CAP-N</u> ¹	<u>691</u>
8	26	++	-	-	-
10	26	+	-	+	-
12	107	+	-	+	-
14	115	+	+	+	-
16	26	+	+	+	+
18	17	ND	+	+	(+)
20	11	ND	+	+	-

¹ A plus (+) indicates immunoreaction was observed between admixed serum and the fusion protein, designated "CAP-N" because it corresponds to the amino terminal of the putative NANBV capsid protein, using the Western blot immunoassay described in Example 4.

The results in Table 2 show immunoreaction between fusion protein and anti-NANBV structural protein antibodies in the sera tested. Furthermore, seroconversion is detectable by the immunoassay using fusion protein containing capsid antigen at times earlier than when the same sera is assayed in the C-100-3-based immunoassay.

In Table 3, results are presented from ALT, anti-HCV and anti-CAP-N assays on sera collected from a human with definitive NANB Hepatitis.

TABLE 3

NYU - 169 - DEFINITIVE NANB HEPATITIS

Week	Post		Anti	
	<u>Infect</u>	<u>ALT</u>	<u>HCV</u>	<u>CAP-N</u>
5	2	34	-	-
	6	8	-	-
	10	150	-	-
10	12	118	-	-
	14	183	-	+
	16	317	-	+
	19	213	-	+
	23	53	-	+

The results in Table 3 show that in the human series 169 seroconversion sera samples, the CAP-N antigen present in the fusion protein detects NANBV-specific antibodies as early as 14 weeks post inoculation, whereas the C-100-3-based immunoassay does not detect any anti-NANBV antibody at the times studied.

In Table 4, results are presented from ALT, EM, anti-HCV, and anti-CAP-N assays on sera from a chimpanzee with a self limited infection presented.

TABLE 4

CHIMP 213 - SELF LIMITED INFECTION

Week	Post		Anti	
	<u>Innoc</u>	<u>ALT</u>	<u>EM</u>	<u>CAP-N</u>
30	4	24	+	+
	6	34	+	+
	8	38	+	+
35	13	28	ND	+

16	25	ND	-	+
18	23	ND	+	+
20	25	-	+	+

The results in Table 4 show that the CAP-N antigen detects anti-NANBV antibodies earlier than the C-100-3 antigen when using sera sampled during the course of a self-limiting NANBV infection.

In Table 5, results are presented from ALT, anti-HCV and anti-CAP-N assays on sera from a chimpanzee that converted from an acute infection profile to a chronic one.

TABLE 5

CHIMP 10 - ACUTE/CHRONIC NANB HEPATITIS

	Week		Peak	Anti	Anti
	Post	Innoc			
<u>Symptoms</u>			<u>ALT</u>	<u>HCV</u>	<u>CAP-N</u>
acute	2		223	-	+
chronic	40		223	+	+
chronic	42		223	+	+
chronic	44		223	+	+
chronic	51		223	+	-

The results in Table 5 indicate that the CAP-N antigen preferentially detects anti-NANBV antibodies in acute stages of NANBV infection.

In Table 6, results are presented from ALT, EM, anti-HCV and anti-Cap-N assays on sera collected at various intervals from several chimpanzees with acute or chronic NANB Hepatitis.

TABLE 6

ADDITIONAL ACUTE SERA

Week	Week
------	------

Post Innoc	Post Alt Elev	Peak ALT	Anti HCV	Anti CAP-N
2	+1	73	-	+
14	+2	66	-	+
5 6	+2	197	-	+
11	+1	151	-	-
8	+4	125	-	+
15	+1	82	-	+
12	-4	73	ND	+

ADDITIONAL CHRONIC SERA

156	+131	110	+	+
156	-	89	+	+
160	-	89	+	+

The results in Table 6 indicate that the CAP-N antigen more often detected anti-NANBV antibodies in sera from acutely infected individuals than did the C-100-3 antigen.

The results of Tables 2-6 show that the NANBV structural protein of the invention, in the form of a fusion protein containing CAP-N antigen and produced by the vector pGEX-3X-690:694, detects antibodies in defined sera conversion series at times in an infected patient or chimpanzee earlier than detectable by present state of the art methods using the C-100-3 antigen. In addition, the results show that CAP-N antigen is particularly useful to detect acute NANBV infection early in the infection.

Taken together, the results indicate that patients infected with NANBV contain circulating antibodies in their blood that are immunospecific for NANBV antigen designated herein as structural antigens, and particularly are shown to immunoreact with the putative capsid antigen defined by CAP-N.

These antibodies are therefore referred to as anti-NANBV structural protein antibodies and are to be distinguished from the class of antibodies previously detected using the NANBV non-structural protein antigen C-100-3.

The foregoing description and the examples are intended as illustrative and are not to be taken as limiting. Still other variations within the spirit and scope of this invention are possible and will readily present themselves to those skilled in the art.

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What Is Claimed Is:

1. A purified DNA segment comprising a nucleotide base sequence that encodes a NANBV structural protein having an amino acid residue sequence that includes an amino acid residue sequence shown in Figure 1 from residue 1 to residue 74.
2. The DNA segment of claim 1 wherein said NANBV structural protein includes an amino acid residue sequence shown in Figure 1 from residue 1 to residue 120.
3. The DNA segment of claim 1 wherein said NANBV structural protein includes an amino acid residue sequence shown in Figure 1 from residue 1 to residue 326.
4. A purified DNA segment comprising a nucleotide base sequence that encodes a NANBV structural protein having an amino acid residue sequence that includes an amino acid residue sequence shown in Figure 1 from residue 69 to residue 120.
5. A purified DNA segment comprising a nucleotide base sequence that encodes a NANBV structural protein having an amino acid residue sequence that includes an amino acid residue sequence shown in Figure 1 from residue 121 to residue 176.
6. The DNA segment of claim 5 wherein said NANBV structural protein include an amino acid residue sequence shown in Figure 1 from residue 121 to residue 326.
7. A recombinant DNA molecule comprising a vector operatively linked to a DNA segment according to claim 1, 4, or 5.
8. The recombinant DNA molecule of claim 7 wherein said vector is an expression vector and said molecule is capable of expressing said protein in a compatible host.

9. The recombinant DNA molecule of claim 8 wherein said molecule encodes a NANBV structural protein having an amino acid residue sequence shown in Figure 2 from residue 1 to residue 316.

10. A transformed cell culture comprising a nutrient medium containing a procaryotic host cell transformed with a recombinant DNA molecule according to claim 8.

11. A method of producing a NANBV structural protein comprising:

a) initiating a culture comprising a nutrient medium containing host cells transformed with a recombinant DNA molecule according to claim 8;

b) maintaining the culture for a time period sufficient for the host cell to express NANBV structural protein; and

c) recovering the NANBV structural protein from the culture.

12. The method of claim 11 wherein said NANBV structural protein has an amino acid residue sequence shown in Figure 2 from residue 1 to residue 316.

13. An isolated NANBV structural protein comprising an amino acid residue sequence shown in Figure 1 from residue 1 to residue 74.

14. The NANBV structural protein of claim 13 wherein said protein includes an amino acid residue sequence shown in Figure 1 from residue 1 to residue 120.

15. The NANBV structural protein of claim 13 wherein said protein includes an amino acid residue sequence shown in Figure 1 from residue 1 to residue 326.

16. The NANBV structural protein of claim 13 wherein said protein has an amino acid residue sequence shown in Figure 2 from residue 1 to residue 316.

5 17. An isolated NANBV structural protein comprising an amino acid residue sequence shown in Figure 1 from residue 69 to residue 120.

18. An isolated NANBV structural protein comprising an amino acid residue sequence shown in Figure 1 from residue 121 to residue 176.

10 19. The NANBV structural protein of claim 18 wherein said protein includes an amino acid residue sequence shown in Figure 1 from residue 121 to residue 326.

15 20. A composition comprising the NANBV structural protein of claim 13, 17, or 18.

20 21. A diagnostic system, in kit form, for assaying a body fluid sample for the presence of antibodies against NANBV structural antigens comprising, in an amount sufficient to perform at least one assay, a NANBV structural protein according to claim 13, 17 or 18.

25 22. The diagnostic system according to claim 21 wherein said NANBV structural protein is affixed to a solid matrix and has an amino acid residue sequence represented by Figure 2 from residue 1 to residue 316.

30 23. A diagnostic system, in kit form, for assaying a body fluid sample for the presence of NANBV structural antigens comprising, in an amount sufficient to perform at least one assay, an anti-NANBV structural protein antibody, said antibody having the capacity to immunoreact with a NANBV structural protein according to claim 13, 17 or 18.

24. The diagnostic system of claim 23 wherein said antibody is affixed to a solid matrix.

25. The diagnostic system of claim 23 that further includes, in an amount sufficient to perform at least one assay, a labeled NANBV structural protein according to claim 13.

26. The diagnostic system of claim 23 that further includes, in an amount sufficient to perform at least one assay, a labeled NANBV structural protein according to claim 17.

27. The diagnostic system of claim 23 that further includes, in an amount sufficient to perform at least one assay, a labeled NANBV structural protein according to claim 18.

28. A method of assaying a body fluid sample for the presence of antibodies against a NANBV structural antigen, which method comprises:

a) forming an immunoreaction admixture by admixing said body fluid sample with a NANBV structural protein, said protein including an amino acid residue sequence represented by the sequence shown in Figure 1 from residue 1 to residue 74, from residue 69 to residue 120, or from residue 121 to residue 176;

b) maintaining said immunoreaction admixture for a time period sufficient for any of said antibodies present to immunoreact with said NANBV structural protein to form an immunoreaction product; and

c) detecting the presence of any of said immunoreaction product from and thereby the presence of said antibodies.

29. The method of claim 28 wherein said NANBV structural protein has an amino acid

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1-100

residue sequence shown in Figure 2 from residue 1 to residue 316.

30. The method of claim 28 wherein said NANBV structural protein is affixed to a solid matrix.

31. The method of claim 30 wherein said detecting in step (c) comprises the steps of:

(i) admixing said immunoreaction product formed in step (c) with a labeled specific binding agent to form a labeling admixture, said labeled specific binding agent comprising a specific binding agent and a label;

(ii) maintaining said labeling admixture for a time period sufficient for any of said immunoreaction product present to bind with said labeled specific binding agent to form a labeled product; and

(iii) detecting the presence of any of said labeled product formed, and thereby the presence of said immunoreaction product.

32. The method of claim 31 wherein said specific binding agent is Protein A.

33. The method of claim 31 wherein said specific binding agent is at least one of the antibodies anti-human IgG and anti-human IgM.

34. The method of claim 31 wherein said label is lanthanide chelate.

35. The method of claim 31 wherein said label is biotin.

36. The method of claim 31 wherein said label is an enzyme.

37. The method of claim 31 wherein said label is a radioactive isotope.

38. A vaccine comprising an immunologically effective amount of a NANBV structural

protein according to claims 13, 17, or 18 in a pharmaceutically acceptable carrier.

39. The vaccine of claim 38 wherein said NANBV structural protein has an amino acid residue sequence represented by Figure 2 from residue 226 to residue 316.

40. A method for inducing immunity to NANBV infection comprising administering an inoculum comprising an immunologically effective amount of the NANBV structural protein according to claim 13, 17 or 18, in a pharmaceutically acceptable carrier.

PATENT APPLICATION DECLARATION AND POWER OF ATTORNEY

I HEREBY DECLARE THAT:

My residence, post office address, and citizenship are as stated next to my name in PART A on page 2 hereof.

I believe I am the original, first, and sole inventor (if only one name is listed) or an original, first, and joint inventor (if plural names are listed) of the subject matter which is claimed and for which a patent is sought on the invention entitled NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

the specification of which:

☒ Is attached hereto
☐ was filed on _____ as Application Serial No. _____
and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Sec. 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Sec. 119 of any foreign application(s) for patent or inventor's certificate listed in PART B on page 2 hereof and have also identified in PART B on page 2 hereof any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

I hereby claim the benefit under Title 35, United States Code, Sec. 120 of any United States application(s) listed in PART C on page 2 hereof and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Sec. 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Sec. 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following as my attorneys or agents with full power of substitution to prosecute this application and transact all business in the United States Patent and Trademark Office connected therewith:

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Stephen D. Geimer	Reg. No. 28,846	John W. Harbst	Reg. No. 28,018	Allen J. Hoover	Reg. No. 24,103
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Gerson E. Meyers	Reg. No. 21,160	John P. Milnamow	Reg. No. 20,635	Paul M. Odell	Reg. No. 28,332
Jack Shore	Reg. No. 17,551	Joel E. Siegel	Reg. No. 25,440	Joseph M. Sorrentino	Reg. No. 32,598
Steven J. Soucar	Reg. No. 32,440	John P. Sumner	Reg. No. 33,039	Marshall W. Sutker	Reg. No. 19,962
Paul M. Vargo	Reg. No. 29,116	Lois P. Besanko	Reg. No. 27,855	Thomas Fitting	Reg. No. P34,163

whose mailing address for this application is: DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAMOW, LTD.
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See Page 2 attached, signed, and made a part hereof.

PATENT APPLICATION DECLARATION AND POWER OF ATTORNEY

PART A: Inventor Information And Signature

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Fourth Inventor's signature: _____ Date: _____

Full name of FIFTH joint inventor, if any _____
Citizenship _____ Residence _____
Post Office Address (If different) _____
Fifth Inventor's signature: _____ Date: _____

PART B: Prior Foreign Application(s)

Serial No.	Country	Day/Month/Year Filed	Priority Claimed
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

PART C: Claim For Benefit Of Filing Date Of Earlier U.S. Application(s)

Serial No.	Filing Date	Status:
		<input type="checkbox"/> Patented <input type="checkbox"/> Pending <input type="checkbox"/> Abandoned
		<input type="checkbox"/> Patented <input type="checkbox"/> Pending <input type="checkbox"/> Abandoned

See Page 1 to which this is attached and from which this Page 2 continues.

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1 ATGAGCACGATTCCCAAACCTCAAAGAAAAACCAAACGTAACACCAAC
MetSerThrIleProLysProGlnArgLysThrLysArgAsnThrAsn 16

49 CGTCGCCACAGGACGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGT
ArgArgProGlnAspValLysPheProGlyGlyGlyGlnIleValGly 32

97 GGAGTTTACTTGTGTCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCG
GlyValTyrLeuLeuProArgArgGlyProArgLeuGlyValArgAla 48

145 ACGAGGAAGACTTCCGAGCGGTGCAACCTCGAGGTAGACGTGAGCCT
ThrArgLysThrSerGluArgSerGlnProArgGlyArgArgGlnPro 64

193 ATCCCCAAGGCACGTGCGCCCGAGGGCAGGACCTGGGCTCAGCCCGG
IleProLysAlaArgArgProGluGlyArgThrTrpAlaGlnProGly 80

241 TACCCTTGCGCCCTCTATGGCAATGAGGGTTGCGGGTGGGCGGGATGG
TyrProTrpProLeuTyrGlyAsnGluGlyCysGlyTrpAlaGlyTrp 96

289 CTCCTGTCTCCCGTGGCTCTCGGCCTAGCTGGGGCCCCACAGACCCC
LeuLeuSerProArgGlySerArgProSerTrpGlyProThrAspPro 112

337 CGGCGTAGGTGCGCGCAATTTGGGTAAGGTCATCGATACCCTTACGTGC
ArgArgArgSerArgAsnLeuGlyLysValIleAspThrLeuThrCys 128

385 GGCTTCGCGCACCTCATGGGTACATACCGCTCGTCGGCGCCCTCTT
GlyPheAlaHisLeuMetGlyTyrIleProLeuValGlyAlaProLeu 144

433 GGAGGCCGTGCCAGGGCCCTGGCGCATGGCGTCCGGGTCTGGAAGAC
GlyGlyArgAlaArgAlaLeuAlaHisGlyValArgValLeuGluAsp 160

481 GCGGTGAACATATGCAACAGGGAACCTTCTGTTGCTCTTTCTCTATC
GlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIle 176

529 TTCCTTCTGGCCCTGCTCTCTTGCCTGACTGTGCGCGCTTACGCCTAC
PheLeuLeuAlaLeuLeuSerCysLeuThrValProAlaSerAlaTyr 192

577 CAAGTGCAGCAATTCCTCAGGGCTTACCAGGTCACCAATGATTGCCCT
GlnValArgAsnSerSerGlyLeuTyrGlnValThrAsnAspCysPro 208

625 AATTCGAGTATTGTGTACGAGGCGCGCATGCCATCCTGCACACTCCG
AsnSerSerIleValTyrGluAlaAlaAspAlaIleLeuHisThrPro 224

673 GGGTGTGTCCCTTGCCTTCGCGAGGGTAACGCCTCGAGGTGTTGGGTG
GlyCysValProCysValArgGluGlyAsnAlaSerArgCysTrpVal 240

721 GCGGTGACCCCCACGGTGGCCACCAGGGACGGCAAACCTCCCCACAACG
AlaValThrProThrValAlaThrArgAspGlyLysLeuProThrThr 256

769 CAGCTTCGACGTCATATCGATCTGCTTGTGCGGAGCGCCACCCTCTGC
GlnLeuArgArgHisIleAspLeuLeuValGlySerAlaThrLeuCys 272

FIGURE 1

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817 TCGGCCCTCTACGTGGGGGACCTGTGCGGGTCTGTCTTTCTCGTTGGT
SerAlaLeuTyrValGlyAspLeuCysGlySerValPheLeuValGly 288

865 CAACTGTTTACCTTCTCTCCCAGGCGCCACTGGACGACGCAAGACTGC
GlnLeuPheThrPheSerProArgArgHisTrpThrThrGlnAspCys 304

913 AATTGTTCTATCTATCCCGGCCATATAACGGGTCATCGCATGGCATGG
AsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrp 320

961 GATATGATGATGAACTGG
AspMetMetMetAsnTrp 326

FIGURE 1 (CONT.)

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1 ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCC
MetSerProIleLeuGlyTyrTrpLysIleLysGlyLeuValGlnPro 16

49 ACTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTTG
ThrArgLeuLeuLeuGluTyrLeuGluGluLysTyrGluGluHisLeu 32

97 TATGAGCCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTG
TyrGluArgAspGluGlyAspLysTrpArgAsnLysLysPheGluLeu 48

145 GGTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAA
GlyLeuGluPheProAsnLeuProTyrTyrIleAspGlyAspValLys 64

193 TTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAAC
LeuThrGlnSerMetAlaIleIleArgTyrIleAlaAspLysHisAsn 80

241 ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAA
MetLeuGlyGlyCysProLysGluArgAlaGluIleSerMetLeuGlu 96

289 GGAGCGGTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGT
GlyAlaValLeuAspIleArgTyrGlyValSerArgIleAlaTyrSer 112

337 AAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAA
LysAspPheGluThrLeuLysValAspPheLeuSerLysLeuProGlu 128

385 ATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAAT
MetLeuLysMetPheGluAspArgLeuCysHisLysThrTyrLeuAsn 144

433 GGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGAT
GlyAspHisValThrHisProAspPheMetLeuTyrAspAlaLeuAsp 160

481 GTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAATTA
ValValLeuTyrMetAspProMetCysLeuAspAlaPheProLysLeu 176

529 GTTTGTTTTAAAAACGTATTGAAGCTATCCCACAAATTGATAAGTAC
ValCysPheLysLysArgIleGluAlaIleProGlnIleAspLysTyr 192

577 TTGAAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCC
LeuLysSerSerLysTyrIleAlaTrpProLeuGlnGlyTrpGlnAla 208

625 ACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGATCTGATCGAAGGT
ThrPheGlyGlyGlyAspHisProProLysSerAspLeuIleGluGly 224

673 CGTGGGATCCCAATTTCGAGCTCGGTACCCATGAGCAGGATTCCCAA
ArgGlyIleProAsnSerSerSerValProMetSerThrIleProLys 240

721 CCTCAAAGAAAAACCAACGTAACACCAACCGTCGCCCACAGGACGTC
ProGlnArgLysThrLysArgAsnThrAsnArgArgProGlnAspVal 256

769 AAGTTCCCGGGTGGCGGTCAGATCGTTGGTGGAGTTTACTTGTGCGG
LysPheProGlyGlyGlyGlnIleValGlyGlyValTyrLeuLeuPro 272

FIGURE 2

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817 CGCAGGGGCCCTAGATTGGGTGTGCGCGACGAGGAAGACTTCCGAG
ArgArgGlyProArgLeuGlyValArgAlaThrArgLysThrSerGlu 288

865 CGGTCGCAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGCACGTCGG
ArgSerGlnProArgGlyArgArgGlnProIleProLysAlaArgArg 304

913 CCCGAGGGCAGGACGGGGATCGGGAATTCATCGTGA
ProGluGlyArgThrGlyIleGlyAsnSerSerEnd 315

74 21

FIGURE 2 (CONT.)

Certification under 37 CFR 1.10 (if applicable)

8/25/90

Date of deposit

I hereby certify that this Transmittal letter, enclosed application, and any other documents referred to as enclosed herein are being deposited in an envelope with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

THOMAS FITTING

(Typed or printed name of person mailing application)

Thomas Fether

(Signature of person mailing application)

COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the utility patent application of inventor(s): Suzanne Zebedee, Genevieve Inchaussepe, Marc S. Nasoff and Alfred M. Prince

and entitled: NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

1. Enclosed are:

- ☒ A duplicate copy of this transmittal letter.
- ☒ One stamped, self-addressed postcard for the PTO Mail Room date stamp.
- ☒ One utility patent application containing pages 1 - 80, and
- ☒ a declaration or oath for the utility patent application including a power of attorney, and
- ☒ drawings: ☐ 1 copy of _____ sheets of formal drawings, OR
- ☒ 1 copy of 4 sheets of informal drawings, OR
- ☐ 1 set of _____ Bristol board sheets of original, formal drawings.
- ☐ A certified copy of a _____ application, No. _____
- ☐ An associate power of attorney.
- ☐ An Information Disclosure Statement.
- ☐ _____ Verified Statement(s) relating to small entity status.
- ☐ Other: _____

2. The filing fee has been calculated as shown below:

	(Col. 1)	(Col. 2)
FOR:	NO. FILED	NO. EXTRA
BASIC FEE		
TOTAL CLAIMS	40 - 20	* 20
INDEP. CLAIMS	15 - 3	* 12
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENTED		

If the difference in Col. 1 is less than zero,
enter "0" in Col. 2.

SMALL ENTITY	
RATE	FEE
	\$ 185
x \$ 6 =	\$
x \$ 18 =	\$
+ \$ 60 =	\$
TOTAL	\$

Q

Q

Q

9.

Q

OTHER THAN A SMALL ENTITY	
RATE	FEE
	\$ 370
x \$ 12 =	\$ 240
x \$ 36 =	\$ 432
+ \$ 120 =	\$
TOTAL	\$ 1,042

Q

Q

Q

9.

Q

- ☐ Please charge my Deposit Account No. 04-1644 in the amount of \$ _____.
- ☒ A check in the amount of \$ 1042.00 to cover the filing fee is enclosed.
- ☒ The Commissioner is authorized to charge payment of the following amounts associated with this communication or credit any overpayment to Deposit Account No. 04-1644:
- ☒ Additional filing fees under 37 CFR 1.16 or deficiencies in remittances therefor.
- ☒ Additional processing fees under 37 CFR 1.17 or deficiencies in remittances therefor.
- ☒ Any deficiency in any patent issue fee under 37 CFR 1.18 for which partial payment is made.

3. The enclosed utility patent application is related to

Date: August 25, 1990

Attorney's Signature _____
Name and Reg. No. _____

Thos. G. Fitts

Correspondence Address:

DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAMOW, LTD.
 11300 Sorrento Valley Road, Suite 200
 San Diego, California 92121
 619/546-1555

THOMAS FITTING, Reg No. P-34,163



**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
07/573,643	08/27/90	SUZANNE ZEBEDEE, et al.	

Thomas Fitting
DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAMOW, LTD.
11300 Sorrento Valley Road, Suite 200
San Diego, CA 92121

EXAMINER	
ART UNIT	PAPER NUMBER
	2

DATE MAILED: Sept 13, 1990

**IF NO RESPONSE TO THIS NOTICE IS RECEIVED WITHIN FORTY-FIVE DAYS, A
FORMAL REQUIREMENT WILL BE ISSUED**

The subject matter of this application appears to:

☒ be "useful in the production or utilization of special nuclear material or atomic energy" as recited in 42 U.S.C. 2182 (Department of Energy (DOE)).

☐ "have significant utility in the conduct of aeronautical and space activities" as recited in 42 U.S.C. 2457 (National Aeronautics and Space Administration (NASA)).

Accordingly, no patent can issue on this application unless applicant(s) file a statement (under oath or in the form of a declaration as provided by 37 CFR 1.68) setting forth (1) the full facts concerning the circumstances under which the invention was made and conceived and (2) the relationship (if any) of the invention to the performance of any work under any contract or other arrangement with the Agency (ies) noted above. On the reverse side of this form is an example of an acceptable format for this statement. The language appearing in paragraphs III and/or IV of the example *must* appear if applicant is attempting to establish that no relationship (under item 2 above) exists.

If the invention disclosed in this application was developed under a contract, grant or cooperative agreement between the Agency indicated above and a person, small business or non-profit organization and rights to the invention have been determined by specific reference to 35 U.S.C. 202 in the contract, grant or cooperative agreement, then applicant need not submit the statement described above. Instead, applicant may file a verified statement (under oath or in the form of a declaration, 37 CFR 1.68) setting forth the information required by 35 U.S.C. 202(c)(6).

IF NO STATEMENT HAS BEEN RECEIVED WITHIN FORTY-FIVE DAYS OF THE MAIL DATE INDICATED ABOVE, a formal requirement for statement will then be issued. No provision is made for extension of the statutory thirty-day period for response to the formal requirement and the penalty for failure to file an acceptable and timely statement is abandonment of the application. Therefore, applicants are strongly encouraged to submit a statement at this time in order to avoid the issuance of a formal requirement.

IT IS IMPORTANT TO NOTE that the statement must accurately represent the property rights situation of the claimed invention if and when the application is found allowable. Thus, if during prosecution before the examiner, the claimed invention is so altered or the property rights situation so changed as to impact the accuracy of a statement submitted earlier, a supplemental statement must be filed. Failure to submit such additional information where appropriate may be considered a false representation of material facts and render the patent owner vulnerable to loss of patent rights and other sanctions as set forth in the statutes. The PTO will not review allowed applications for this possibility. The responsibility for complying with the statutes rests with the applicants.

Any questions regarding this requirement should be directed to Licensing and Review at (703) 557-3011.

**PLEASE DIRECT ALL COMMUNICATIONS RELATING TO THIS MATTER TO THE
ATTENTION OF LICENSING AND REVIEW**



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
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07/573,643 08/27/90 ZEBEDEE

S

PHA0025P

DRESSLER, GOLDSMITH, SHORE,
SUTKER & MILNAMOW, LTD.
11300 SORRENTO VALLEY RD., STE. 200
SAN DIEGO, CA 92121

000

DATE MAILED:

10/02/90

NOTICE TO FILE MISSING PARTS OF APPLICATION—
FILING DATE GRANTED

A filing date has been granted to this application. However, the following parts are missing.

If all missing parts are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 362.00

1. ☐ The statutory basic filing fee is: ☐ missing, ☐ insufficient. Applicant as a ☐ large entity, ☐ small entity, must submit \$ _____ to complete the basic filing fee and MUST ALSO SUBMIT THE SURCHARGE AS INDICATED BELOW.

2. ☒ Additional claim fees of \$ 240.00 as a ☒ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due. NO SURCHARGE IS REQUIRED FOR THIS ITEM.

3. ☐ The oath or declaration:

☐ is missing.

☐ does not cover items omitted at the time of execution.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Serial Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63 identifying the application by the above Serial Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

5. ☒ The signature to the oath or declaration is: ☐ missing, ☐ a reproduction, ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Serial Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration: _____ Applicant(s) should provide, if possible, an oath or declaration signed by the omitted inventor(s), identifying this application by the above Serial Number and Filing Date. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$26.00 under 37 CFR 1.17(k), unless this fee has already been paid. NO SURCHARGE UNDER 37 CFR 1.16(e) IS REQUIRED FOR THIS ITEM.

8. ☐ A \$20.00 processing fee is required for returned checks. (37 CFR 1.21(m)).

9. ☐ Your filing receipt was mailed in error because check was returned.

10. ☐ Other:

A Serial Number and Filing Date have been assigned to this application. However, to avoid abandonment under 37 CFR 1.53(d), the missing parts and fees identified above in items 1 and 3-6 must be timely provided ALONG WITH THE PAYMENT OF A SURCHARGE OF \$110.00 for large entities or \$55.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e). Applicant is given ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE of this application, WHICHEVER IS LATER, within which to file all missing parts and pay any fees. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

Direct the response to, and any questions about, this notice to the undersigned, Attention: Application Branch.

A copy of this notice **MUST** be returned with response.

For: Manager, Application Branch
(703) 557-3254

For Office Use Only

<input type="checkbox"/> 102	<input type="checkbox"/> 202
<input type="checkbox"/> 103	<input type="checkbox"/> 203
<input type="checkbox"/> 104	<input type="checkbox"/> 204
<input type="checkbox"/> 105	<input type="checkbox"/> 205

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee, et al.)
Serial No.: ~~07/548,027~~) Group Art Unit: Unassigned
573,643)
Filed: July 5, 1990) Examiner: Unassigned
FOR: NON-A, NON-B HEPATITIS VIRUS)
ANTIGEN, DIAGNOSTIC METHOD)
AND VACCINES)



Our Ref. No. PHA 0025P
San Diego, California
October 25, 1990

COMMUNICATION

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231


Sir:

In response to the Notice of Missing Parts of Application under 37 CFR §1.53(d) dated October 2, 1990, enclosed is a Declaration and Power of Attorney signed by the applicants that refers to the above-identified application.

Enclosed is Check No. 3452 in the amount of \$360.00 to cover the surcharge set forth in CFR §1.16(e).

Please charge any additional fees concerning this matter to our Deposit Account No. 04-1644.

Respectfully submitted,

By 
Douglas A. Bingham
Reg. No. 32,457

DRESSLER, GOLDSMITH, SHORE,
SUTKER & MILNAMOW, LTD.
11300 Sorrento Valley Road
Suite 200
San Diego, California 92121
(619) 546-1555

RECEIVED

NOV 06 1990


APPLICATION DIVISION 405

CERTIFICATE OF MAILING

I hereby certify that this COMMUNICATION is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on the date written below.

26 Oct 90
Date

Douglas A. Bingham
Typed or Printed Name of
Person Mailing Correspondence


Signature of Person Mailing Correspondence

PATENT APPLICATION DECLARATION AND POWER OF ATTORNEY

I HEREBY DECLARE THAT:

My residence, post office address, and citizenship are as stated next to my name in PART A on page 2 hereof.

I believe I am the original, first, and sole inventor (if only one name is listed) or an original, first, and joint inventor (if plural names are listed) of the subject matter which is claimed and for which a patent is sought on the invention entitled NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

The specification of which:

☐ is attached hereto
☒ was filed on August 25, 1990 as Application Serial No. 07/573,643
 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Sec. 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Sec. 119 of any foreign application(s) for patent or inventor's certificate listed in PART B on page 2 hereof and have also identified in PART B on page 2 hereof any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

I hereby claim the benefit under Title 35, United States Code, Sec. 120 of any United States application(s) listed in PART C on page 2 hereof and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Sec. 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Sec. 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following as my attorneys or agents with full power of substitution to prosecute this application and transact all business in the United States Patent and Trademark Office connected therewith:

Douglas A. Bingham	Reg. No. <u>32,457</u>	Talivaldis Cepuritis	Reg. No. <u>20,818</u>	Ernest Cheslow	Reg. No. <u>17,019</u>
Max Dressler	Reg. No. <u>14,123</u>	William C. Fuess	Reg. No. <u>30,054</u>	Edward P. Gamson	Reg. No. <u>29,381</u>
Stephen D. Geimer	Reg. No. <u>28,846</u>	John W. Harbst	Reg. No. <u>28,018</u>	Allen J. Hoover	Reg. No. <u>24,103</u>
Henry S. Kaplan	Reg. No. <u>28,346</u>	Martin L. Katz	Reg. No. <u>25,011</u>	John W. Klooster	Reg. No. <u>18,953</u>
Gerson E. Meyers	Reg. No. <u>21,160</u>	John P. Milnamow	Reg. No. <u>20,635</u>	Paul M. Odell	Reg. No. <u>28,332</u>
Jack Shore	Reg. No. <u>17,551</u>	Joel E. Siegel	Reg. No. <u>25,440</u>	Joseph M. Sorrentino	Reg. No. <u>32,598</u>
Steven J. Soucar	Reg. No. <u>32,440</u>	John P. Sumner	Reg. No. <u>33,039</u>	Marshall W. Sutker	Reg. No. <u>19,962</u>
Paul M. Vargo	Reg. No. <u>29,116</u>	Lois P. Besanko	Reg. No. <u>27,855</u>	Thomas Fitting	Reg. No. <u>34,163</u>

whose mailing address for this application is: 601 DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAMOW, LTD.
602 11300 Sorrento Valley Road, Suite 200
701 San Diego, California 92121
 Telephone: (619) 546-1555

See Page 2 attached, signed, and made a part hereof.

PATENT APPLICATION DECLARATION AND POWER OF ATTORNEY

PART A: Inventor Information And Signature

Full name of SOLE or FIRST inventor Suzanne Zebedee
 Citizenship USA Residence 7544 Charmant Drive
San Diego, California 92122 CA

Post Office Address (If different) _____

Inventor's signature: Suzanne Zebedee Date: 10.23.90

Full name of SECOND joint inventor, if any Genevieve Inchausti
 Citizenship France Residence 504 East 63rd Street
New York, NY 10021

Post Office Address (If different) _____

Second Inventor's signature: _____ Date: _____

Full name of THIRD joint inventor, if any Marc S. Nasoff
 Citizenship USA Residence 11734 Mira Lago Way
San Diego, California 92131 CA

Post Office Address (If different) _____

Third Inventor's signature: X Marc S. Nasoff Date: 10/23/90

Full name of FOURTH joint inventor, if any Alfred M. Prince
 Citizenship USA Residence 154 Stone Gill Road, Pound Ridge
New York, NY 10576

Post Office Address (If different) _____

Fourth Inventor's signature: _____ Date: _____

Full name of FIFTH joint inventor, if any _____
 Citizenship _____ Residence _____

Post Office Address (If different) _____

Fifth Inventor's signature: _____ Date: _____

PART B: Prior Foreign Application(s)

Serial No.	Country	Day/Month/Year Filed	Priority Claimed
			<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

PART C: Claim For Benefit Of Filing Date Of Earlier U.S. Application(s)

Serial No.	Filing Date	Status:
		<input type="checkbox"/> Patented <input type="checkbox"/> Pending <input type="checkbox"/> Abandoned
		<input type="checkbox"/> Patented <input type="checkbox"/> Pending <input type="checkbox"/> Abandoned

See Page 1 to which this is attached and from which this Page 2 continues.

PATENT APPLICATION DECLARATION AND POWER OF ATTORNEY

I HEREBY DECLARE THAT:

My residence, post office address, and citizenship are as stated next to my name in PART A on page 2 hereof.

I believe I am the original, first, and sole inventor (if only one name is listed) or an original, first, and joint inventor (if plural names are listed) of the subject matter which is claimed and for which a patent is sought on the invention entitled NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

the specification of which:

☐ is attached hereto
☒ was filed on August 25, 1990 as Application Serial No. 07/573,643
 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Sec. 1.56(a).

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I hereby claim the benefit under Title 35, United States Code, Sec. 120 of any United States application(s) listed in PART C on page 2 hereof and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Sec. 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Sec. 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following as my attorneys or agents with full power of substitution to prosecute this application and transact all business in the United States Patent and Trademark Office connected therewith:

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Stephen D. Geimer	Reg. No. 28,846	John W. Harbst	Reg. No. 28,018	Allen J. Hoover	Reg. No. 24,103
Henry S. Kaplan	Reg. No. 25,346	Martin L. Katz	Reg. No. 25,011	John W. Klooster	Reg. No. 18,953
Gerson E. Meyers	Reg. No. 21,160	John P. Milnamow	Reg. No. 20,635	Paul M. Odell	Reg. No. 28,332
Jack Shore	Reg. No. 17,551	Joel E. Siegel	Reg. No. 25,440	Joseph M. Sorrentino	Reg. No. 32,598
Steven J. Soucar	Reg. No. 32,440	John P. Sumner	Reg. No. 33,039	Marshall W. Sutker	Reg. No. 19,962
Paul M. Vargo	Reg. No. 29,116	Lois P. Besanko	Reg. No. 27,855	Thomas Fitting	Reg. No. 34,163

whose mailing address for this application is: **DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAMOW, LTD.**
11300 Sorrento Valley Road, Suite 200
San Diego, California 92121
Telephone: (619) 546-1555

See Page 2 attached, signed, and made a part hereof.

PATENT APPLICATION DECLARATION AND POWER OF ATTORNEY

PART A: Inventor Information And Signature

Full name of SOLE or FIRST inventor Suzanne Zebedee
 Citizenship USA Residence 7544 Charmant Drive
San Diego, California 92122

Post Office Address (If different) _____

Inventor's signature: _____ Date: _____

402-pp
 Full name of SECOND joint inventor, if any Genevieve Inchauspe
 Citizenship France Residence 504 East 63rd Street
New York, NY 10021 *NY*

Post Office Address (If different) _____

Second Inventor's signature: Genevieve Inchauspe Date: 10/15/90

Full name of THIRD joint inventor, if any Marc S. Nasoff
 Citizenship USA Residence 11734 Mira Lago Way
San Diego, California 92131 *24*

Post Office Address (If different) _____

Third Inventor's signature: _____ Date: _____

404-pp
 Full name of FOURTH joint inventor, if any Alfred M. Prince
 Citizenship USA Residence 154 Stone Gill Road, Pound Ridge
New York, NY 10576 *NY*

Post Office Address (If different) _____

Fourth Inventor's signature: Alfred M. Prince Date: 10-14-90

Full name of FIFTH joint inventor, if any _____
 Citizenship _____ Residence _____

Post Office Address (If different) _____

Fifth Inventor's signature: _____ Date: _____

PART B: Prior Foreign Application(s)

Serial No.	Country	Day/Month/Year Filed	Priority Claimed
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

PART C: Claim For Benefit Of Filing Date Of Earlier U.S. Application(s)

Serial No.	Filing Date	Status:
		<input type="checkbox"/> Patented <input type="checkbox"/> Pending <input type="checkbox"/> Abandoned
		<input type="checkbox"/> Patented <input type="checkbox"/> Pending <input type="checkbox"/> Abandoned

See Page 1 to which this is attached and from which this Page 2 continues.



240-103
120-105
A/N
M

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

90-103-105-106-107-108-109-110-111-112-113-114-115-116-117-118-119-120-121-122-123-124-125-126-127-128-129-130-131-132-133-134-135-136-137-138-139-140-141-142-143-144-145-146-147-148-149-150-151-152-153-154-155-156-157-158-159-160-161-162-163-164-165-166-167-168-169-170-171-172-173-174-175-176-177-178-179-180-181-182-183-184-185-186-187-188-189-190-191-192-193-194-195-196-197-198-199-200-201-202-203-204-205-206-207-208-209-210-211-212-213-214-215-216-217-218-219-220-221-222-223-224-225-226-227-228-229-230-231-232-233-234-235-236-237-238-239-240-241-242-243-244-245-246-247-248-249-250-251-252-253-254-255-256-257-258-259-260-261-262-263-264-265-266-267-268-269-270-271-272-273-274-275-276-277-278-279-280-281-282-283-284-285-286-287-288-289-290-291-292-293-294-295-296-297-298-299-300-301-302-303-304-305-306-307-308-309-310-311-312-313-314-315-316-317-318-319-320-321-322-323-324-325-326-327-328-329-330-331-332-333-334-335-336-337-338-339-340-341-342-343-344-345-346-347-348-349-350-351-352-353-354-355-356-357-358-359-360-361-362-363-364-365-366-367-368-369-370-371-372-373-374-375-376-377-378-379-380-381-382-383-384-385-386-387-388-389-390-391-392-393-394-395-396-397-398-399-400-401-402-403-404-405-406-407-408-409-410-411-412-413-414-415-416-417-418-419-420-421-422-423-424-425-426-427-428-429-430-431-432-433-434-435-436-437-438-439-440-441-442-443-444-445-446-447-448-449-450-451-452-453-454-455-456-457-458-459-460-461-462-463-464-465-466-467-468-469-470-471-472-473-474-475-476-477-478-479-480-481-482-483-484-485-486-487-488-489-490-491-492-493-494-495-496-497-498-499-500-501-502-503-504-505-506-507-508-509-510-511-512-513-514-515-516-517-518-519-520-521-522-523-524-525-526-527-528-529-530-531-532-533-534-535-536-537-538-539-540-541-542-543-544-545-546-547-548-549-550-551-552-553-554-555-556-557-558-559-560-561-562-563-564-565-566-567-568-569-570-571-572-573-574-575-576-577-578-579-580-581-582-583-584-585-586-587-588-589-590-591-592-593-594-595-596-597-598-599-600-601-602-603-604-605-606-607-608-609-610-611-612-613-614-615-616-617-618-619-620-621-622-623-624-625-626-627-628-629-630-631-632-633-634-635-636-637-638-639-640-641-642-643-644-645-646-647-648-649-650-651-652-653-654-655-656-657-658-659-660-661-662-663-664-665-666-667-668-669-670-671-672-673-674-675-676-677-678-679-680-681-682-683-684-685-686-687-688-689-690-691-692-693-694-695-696-697-698-699-700-701-702-703-704-705-706-707-708-709-710-711-712-713-714-715-716-717-718-719-720-721-722-723-724-725-726-727-728-729-730-731-732-733-734-735-736-737-738-739-740-741-742-743-744-745-746-747-748-749-750-751-752-753-754-755-756-757-758-759-760-761-762-763-764-765-766-767-768-769-770-771-772-773-774-775-776-777-778-779-780-781-782-783-784-785-786-787-788-789-790-791-792-793-794-795-796-797-798-799-800-801-802-803-804-805-806-807-808-809-810-811-812-813-814-815-816-817-818-819-820-821-822-823-824-825-826-827-828-829-830-831-832-833-834-835-836-837-838-839-840-841-842-843-844-845-846-847-848-849-850-851-852-853-854-855-856-857-858-859-860-861-862-863-864-865-866-867-868-869-870-871-872-873-874-875-876-877-878-879-880-881-882-883-884-885-886-887-888-889-890-891-892-893-894-895-896-897-898-899-900-901-902-903-904-905-906-907-908-909-910-911-912-913-914-915-916-917-918-919-920-921-922-923-924-925-926-927-928-929-930-931-932-933-934-935-936-937-938-939-940-941-942-943-944-945-946-947-948-949-950-951-952-953-954-955-956-957-958-959-960-961-962-963-964-965-966-967-968-969-970-971-972-973-974-975-976-977-978-979-980-981-982-983-984-985-986-987-988-989-990-991-992-993-994-995-996-997-998-999-1000	FILING DATE: 08/27/90	FIRST NAMED APPLICANT: ZEBEDEE	ATTY. DOCKET NO.: PHA0025P
---	-----------------------	--------------------------------	----------------------------

DRESSLER, GOLDSMITH, SHORE,
SUTKER & MILNAMOW, LTD.
11300 SORRENTO VALLEY RD., STE. 200
SAN DIEGO, CA 92121

000

DATE MAILED:

10/02/90

**NOTICE TO FILE MISSING PARTS OF APPLICATION—
FILING DATE GRANTED**

A filing date has been granted to this application. However, the following parts are missing.

If all missing parts are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 240.00

1. ☐ The statutory basic filing fee is: ☐ missing, ☐ insufficient. Applicant as a ☐ large entity, ☐ small entity, must submit \$_____ to complete the basic filing fee and **MUST ALSO SUBMIT THE SURCHARGE AS INDICATED BELOW.**

2. ☒ Additional claim fees of \$ 240.00 as a ☒ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due. **NO SURCHARGE IS REQUIRED FOR THIS ITEM.**

3. ☐ The oath or declaration:
☐ is missing.
☐ does not cover items omitted at the time of execution.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Serial Number and Filing Date is required. **A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.**

4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63 identifying the application by the above Serial Number and Filing Date is required. **A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.**

5. ☒ The signature to the oath or declaration is: ☒ missing; ☐ a reproduction; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Serial Number and Filing Date is required. **A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.**

6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration: _____ Applicant(s) should provide, if possible an oath or declaration signed by the omitted inventor(s), identifying this application by the above Serial Number and Filing Date. **A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.**

7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$26.00 under 37 CFR 1.17(k), unless this fee has already been paid. **NO SURCHARGE UNDER 37 CFR 1.16(e) IS REQUIRED FOR THIS ITEM.**

8. ☐ A \$20.00 processing fee is required for returned checks. (37 CFR 1.21(m)).

9. ☐ Your filing receipt was mailed in error because check was returned.

10. ☐ Other:

A Serial Number and Filing Date have been assigned to this application. However, to avoid abandonment under 37 CFR 1.53(d), the missing parts and fees identified above in items 1 and 3-6 must be timely provided **ALONG WITH THE PAYMENT OF A SURCHARGE OF \$110.00** for large entities or **\$55.00** for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e). Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all missing parts and pay any fees. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

Direct the response to, and any questions about, this notice to the undersigned, Attention: Application Branch.

A copy of this notice MUST be returned with response.

040-RP 11/02/90 07548027

1:103

240.00 CK

040-RP 11/02/90 07548027

1:105

120.00 CK

For: Manager, Application Branch
(703) 557-3254

COPY TO BE RETURNED WITH RESPONSE

For Office Use Only

<input type="checkbox"/> 102	<input type="checkbox"/> 202
<input type="checkbox"/> 103	<input type="checkbox"/> 203
<input type="checkbox"/> 104	<input type="checkbox"/> 204
<input type="checkbox"/> 105	<input type="checkbox"/> 205

IN THE UNITED STATES PATENT AND TRADE MARK OFFICE

APPLICANT: Zebedee et al.

SERIAL NO.: 07/573,643

FILED: August 25, 1990

FOR: NON-A, NON-B HEPATITIS VIRUS
ANTIGEN, DIAGNOSTIC METHODS
AND VACCINES

GROUP ART UNIT:
Unassigned

EXAMINER:
Unassigned

San Diego, California
Ref. No. PHA0025

NOV 5 1990

LICENSING & REVIEW

COMMUNICATION

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Attn: LICENSING AND
REVIEW

Sir:

Enclosed herewith is a DECLARATION of the inventor of the subject application pursuant to the provision of Section 152 of the Atomic Energy Act, 42 U.S.C. 2182, as amended, filed as a supplemental statement in response to the Office Action mailed September 13, 1990.

It is believed that no processing fee is required to accompany this COMMUNICATION and DECLARATION. However, the Commissioner is hereby authorized to charge such fees as may be necessary in connection with this matter to our Deposit Account No. 04-1644.

Respectfully submitted,

By Thomas Fitting
Thomas Fitting, Reg. No. 34,163

DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAMOW, LTD.
11300 Sorrento Valley Road, Suite 200
San Diego, CA 92121
619/546-1555

CERTIFICATE OF MAILING

I hereby certify that this COMMUNICATION is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Hon. Commissioner of Patents and Trademarks, Attn: Licensing and Review, Washington, DC 20231 on the date indicated below.

Thomas Fitting
Thomas Fitting

26 October, 1990
Date

TF/bj

IN THE UNITED STATES PATENT AND TRADE MARK OFFICE



APPLICANT: Zebedee et al.)
SERIAL NO.: 07/573,643) GROUP ART UNIT:
FILED: August 25, 1990) Unassigned
FOR: NON-A, NON-B HEPATITIS VIRUS) EXAMINER:
ANTIGEN, DIAGNOSTIC METHODS) Unassigned
AND VACCINES) San Diego, California
Ref.No. PHA0025)

RECEIVED

DECLARATION

NOV 5 1990

LICENSING & REVIEW

Hon. Commissioner of Patents and Trademarks
Attn: Licensing and Review
Washington, DC 20231

Sir:

OK
H
We, Suzanne Zebedee, Marc S. Nasoff, and Alfred M. Prince, citizens of the United States of America, presently residing at 7544 Charmant Drive, San Diego, California 92122, 11734 Mira Lago Way, San Diego, California 92131, and 154 Stone Gill Road, Pound Ridge, New York, New York 10576, and Genevieve Inchausepe, a citizen of France, residing at 504 East 63rd Street, New York, New York 10021, respectively, declare:

That we made and conceived the invention described and claimed in patent application Serial No. 07/573,643, filed in the United States of America on August 25, 1990, titled: NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES;

That we made and conceived this invention while employed by Pharmacia Genetic Engineering, Inc.;

That the invention is related to the work we were employed to perform and was made within the scope of our employment duties;

That the invention was made during working hours and with the use of facilities, equipment, materials, funds, information and services of Pharmacia Genetic Engineering, Inc.; and

That the invention was not made or conceived in the course of, or in connection with or under, the terms of any grant, contract, subcontract, or arrangement entered into with or for the benefit of the United States Atomic Energy Commission or its

Serial No. 07/573 13

2

successors: Energy Research and Development Administration or the Department of Energy.

We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing on this application.

Suzanne Zebedee _____ Date

Genevieve Inchausti 10/15/90
Genevieve Inchausti _____ Date

Marc S. Nasoff _____ Date

Alfred M. Price 10-14-90
Alfred M. Price _____ Date

CERTIFICATE OF MAILING

I hereby certify that this DECLARATION is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Hon. Commissioner of Patents and Trademarks, Attn: Licensing and Review, Washington, DC 20231 on the date indicated below.

Thomas Fitting 26 October, 1990
Thomas Fitting, Reg. No. 34,163 _____ Date

DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAMOW, LTD.
11300 Sorrento Valley Road, Suite 200
San Diego, California 92121
619/546-1555

TF/bj

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



APPLICANT: Zebedee et al.)
SERIAL NO.: 07/573,643) GROUP ART UNIT:
FILED: August 25, 1990) Unassigned
FOR: NON-A, NON-B HEPATITIS VIRUS) EXAMINER:
ANTIGEN, DIAGNOSTIC METHODS) Unassigned
AND VACCINES) San Diego, California
Ref.No. PHA0025)

DECLARATION

RECEIVED

NOV 5 1990

LICENSING & REVIEW

Hon. Commissioner of Patents and Trademarks
Attn: Licensing and Review
Washington, DC 20231

Sir:

OK
H
We, Suzanne Zebedee, Marc S. Nasoff, and Alfred M. Prince, citizens of the United States of America, presently residing at 7544 Charmant Drive, San Diego, California 92122, 11734 Mira Lago Way, San Diego, California 92131, and 154 Stone Gill Road, Pound Ridge, New York, New York 10576, and Genevieve Inchausepe, a citizen of France, residing at 504 East 63rd Street, New York, New York 10021, respectively, declare:

That we made and conceived the invention described and claimed in patent application Serial No. 07/573,643, filed in the United States of America on August 25, 1990, titled: NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES;

That we made and conceived this invention while employed by Pharmacia Genetic Engineering, Inc.;

That the invention is related to the work we were employed to perform and was made within the scope of our employment duties;

That the invention was made during working hours and with the use of facilities, equipment, materials, funds, information and services of Pharmacia Genetic Engineering, Inc.; and

That the invention was not made or conceived in the course of, or in connection with or under, the terms of any grant, contract, subcontract, or arrangement entered into with or for the benefit of the United States Atomic Energy Commission or its

Serial No. 07/573,643

2

successors: Energy Research and Development Administration or the Department of Energy.

We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing on this application.

Suzanne Zebedee 10.23.90
Date

Genevieve Inchausepe Date

Marc S. Nasoff 10/23/90
Date

Alfred M. Price Date

CERTIFICATE OF MAILING

I hereby certify that this DECLARATION is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Hon. Commissioner of Patents and Trademarks, Attn: Licensing and Review, Washington, DC 20231 on the date indicated below.

Thomas Fitting 26 October, 1990
Thomas Fitting, Reg. No. 34,163 Date

DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAMOW, LTD.
11300 Sorrento Valley Road, Suite 200
San Diego, California 92121
619/546-1555

TF/bj

Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
TRADEMARKS

573643
SERIAL NO (SERIES OF 1970)

#6

PTO-103c (rev. 6-79) SECURITY FILE	GROUP NO.	FILING DATE		INVENTION	
	APPLICANT				
SCREENED BY					
LICENSE <input type="checkbox"/>					
DATE					
DARCOM <input type="checkbox"/>	DRAWINGS	TOTAL CL'S	IND CL'S	FILING FEE REC	TRANSACTION
NAVY <input type="checkbox"/>	DOE				
AF <input type="checkbox"/>					
CE <input type="checkbox"/>					
DOE <input type="checkbox"/>					
NASA <input type="checkbox"/>					

JAN 0 8 1991

ACCESS ACKNOWLEDGEMENT

As Required by
Title 35, United States Code (1952) Section 181

I hereby acknowledge that I have inspected the disclosure of the above identified application for patent in the administration of the law cited above, on behalf of the department or agency which I represent, and promise that any information acquired from said application will not be divulged, disclosed or used for any purpose other than in the administration of the cited law.

NAME	DATE	AGENCY REPRESENTED
V. Carson	1-4-91	DOE



120.00 - 124

DAE
#7

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Zebedee et al.

SERIAL NO.: 07/573,643

FILED: August 27, 1990

FOR: NON-1, NON-B HEPATITIS VIRUS
ANTIGEN, DIAGNOSTIC METHODS
AND VACCINES

GROUP ART UNIT: Unassigned

EXAMINER: Unassigned

San Diego, California
Ref. No. PHA0025

RECEIVED
NOV 19 1990
OFFICE OF THE
COMMISSIONER
OF PATENTS

PETITION UNDER RULE 1.182

Hon. Commissioner of Patents and Trademarks
Washington, DC 20231

Attn: Application Division

RECEIVED

NOV 16 1990

DEPUTY ASST. COMM.

Sir:

It is respectfully requested that the filing date afforded the above-identified application be corrected from the filing date of August 27, 1990 as shown on Form PTOL-456, a copy of which is enclosed as Exhibit I.

The above-identified application was filed by Express Mail on August 25, 1990 as evidenced by the additional Exhibits. The application should, therefore, have been granted a filing date of August 25, 1990 rather than August 27, 1990, as is shown in the enclosed Exhibit I.

In particular, Exhibit II is a photocopy of the transmittal letter that accompanied the filing of the application. The Certificate of Express Mailing on the top of the transmittal letter lists the Express Mail label number (RB212458125) and the name and signature of the person who mailed the application (Thomas Fitting). The transmittal letter is signed by counsel who filed the application, Thomas Fitting, Reg. No. 34,163) and has the same date of mailing.

120 LA 11/13/90 07573643

Exhibit III is a photocopy of the receipt of the Express Mail label used to file the application. The label number on this receipt corresponds to the label number on the Certificate of Express Mailing in Exhibit II, and the proper addressee is also shown, as is the correct postage for the Express Mail package.

Exhibit III further shows that a Postal Service clerk with the initials JJ or JF at a United States Post Office in Zip Code Region 92110 received the application on August 25, 1990. Thus, the application was indeed deposited on August 25, 1990, the date of receipt indicated on the Express Mail label by the Postal Service clerk.

It is submitted that the above discussion and the enclosed Exhibits I-III, attached hereto, clearly show that the application was in fact filed on August 25, 1990. The transmittal letter bears the date of August 25, 1990, and the Express Mail receipt shows that the application was deposited at a United States Post Office and received by a U.S. Postal clerk during business hours on August 25, 1990. Thus, pursuant to 37 C.F.R. §1.10(c), the application was filed on August 25, 1990.

In view of the fact that the application was actually filed on August 25, 1990, it is further respectfully petitioned that the application be afforded a filing date of August 25, 1990 on all of the documents in the file.

Check No. 3437 in the amount of \$120.00 is enclosed for this Petition fee. Please charge our Deposit Account No. 04-1644 should any further fee be needed.

Serial No. 07/573,643

3

Respectfully submitted,

By Thomas Fitting
Thomas Fitting, Reg. No. 34,163

Enclosures
Exhibits I-III
Postcard

DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAMOW, LTD.
11300 Sorrento Valley Road, Suite 200
San Diego, California 92121
619/546-1555

CERTIFICATE OF MAILING

I hereby certify that this PETITION and the enclosures are being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Hon. Commissioner of Patents and Trademarks, Washington, DC 20231, Attn: Application Division, on the date as indicated below.

Thomas Fitting October 16, 1990
Thomas Fitting Date

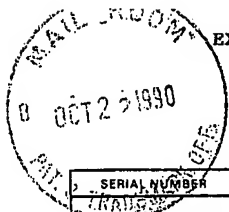


EXHIBIT I

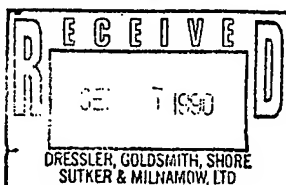


**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
07/573,643	08/27/90	SUZANNE ZEBEDEE, et al.	

Thomas Fitting
DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAMOW, LTD.
11300 Sorrento Valley Road, Suite 200
San Diego, CA 92121



9/20/90

DOCKETED	EXAMINER
ART UNIT	PAPER NUMBER
	2

DATE MAILED: Sept 13, 1990

**IF NO RESPONSE TO THIS NOTICE IS RECEIVED WITHIN FORTY-FIVE DAYS, A
FORMAL REQUIREMENT WILL BE ISSUED**

The subject matter of this application appears to:

☒ be "useful in the production or utilization of special nuclear material or atomic energy" as recited in 42 U.S.C. 2182 (Department of Energy (DOE)).

☐ "have significant utility in the conduct of aeronautical and space activities" as recited in 42 U.S.C. 2457 (National Aeronautics and Space Administration (NASA)).

Accordingly, no patent can issue on this application unless applicant(s) file a statement (under oath or in the form of a declaration as provided by 37 CFR 1.68) setting forth (1) the full facts concerning the circumstances under which the invention was made and conceived and (2) the relationship (if any) of the invention to the performance of any work under any contract or other arrangement with the Agency(ies) noted above. On the reverse side of this form is an example of an acceptable format for this statement. The language appearing in paragraphs III and/or IV of the example *must* appear if applicant is attempting to establish that no relationship (under item 2 above) exists.

If the invention disclosed in this application was developed under a contract, grant or cooperative agreement between the Agency indicated above and a person, small business or non-profit organization and rights to the invention have been determined by specific reference to 35 U.S.C. 202 in the contract, grant or cooperative agreement, then applicant need not submit the statement described above. Instead, applicant may file a verified statement (under oath or in the form of a declaration, 37 CFR 1.68) setting forth the information required by 35 U.S.C. 202(c)(6).

IF NO STATEMENT HAS BEEN RECEIVED WITHIN FORTY-FIVE DAYS OF THE MAIL DATE INDICATED ABOVE, a formal requirement for statement will then be issued. No provision is made for extension of the statutory thirty-day period for response to the formal requirement and the penalty for failure to file an acceptable and timely statement is abandonment of the application. Therefore, applicants are strongly encouraged to submit a statement at this time in order to avoid the issuance of a formal requirement.

IT IS IMPORTANT TO NOTE that the statement must accurately represent the property rights situation of the claimed invention if and when the application is found allowable. Thus, if during prosecution before the examiner, the claimed invention is so altered or the property rights situation so changed as to impact the accuracy of a statement submitted earlier, a supplemental statement must be filed. Failure to submit such additional information where appropriate may be considered a false representation of material facts and render the patent owner vulnerable to loss of patent rights and other sanctions as set forth in the statutes. The PTO will not review allowed applications for this possibility. The responsibility for complying with the statutes rests with the applicants.

Any questions regarding this requirement should be directed to Licensing and Review at (703) 557-3011.

**PLEASE DIRECT ALL COMMUNICATIONS RELATING TO THIS MATTER TO THE
ATTENTION OF LICENSING AND REVIEW**

Certification under 37 CFR 1.10 (if applicable)

RB212458125

8/25/90

"Express Mail" mailing label number

Date of deposit

I hereby certify that this Transmittal letter, enclosed application, and any other documents referred to as enclosed herein are being deposited in an envelope with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

THOMAS FITTING

(Typed or printed name of person mailing application)

Thomas Fitting

(Signature of person mailing application)

COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the utility patent application of inventor(s): Suzanne Zebedee,Genevieve Inchaussepe, Marc S. Nasoff and Alfred M. Princeand entitled: NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

1. Enclosed are:

- ☒ A duplicate copy of this transmittal letter.
☒ One stamped, self-addressed postcard for the PTO Mail Room date stamp.
☒ One utility patent application containing pages 1 - 80, and
☒ a declaration or oath for the utility patent application including a power of attorney, and
☒ drawings: ☐ 1 copy of _____ sheets of formal drawings, OR
☒ 1 copy of 4 sheets of informal drawings, OR
☐ 1 set of _____ Bristol board sheets of original, formal drawings.
☐ A certified copy of a _____ application, No. _____
☐ An associate power of attorney.
☐ An Information Disclosure Statement.
☐ _____ Verified Statement(s) relating to small entity status.
☐ Other: _____

2. The filing fee has been calculated as shown below:

(Col. 1)		(Col. 2)	SMALL ENTITY		OTHER THAN A SMALL ENTITY	
FOR:	NO. FILED	NO. EXTRA	RATE	FEE	RATE	FEE
BASIC FEE				\$ 185		\$ 370
TOTAL CLAIMS	40 - 20	* 20	x \$ 6 =	\$	x \$ 12 =	\$ 240
INDEP. CLAIMS	15 - 3	* 12	x \$ 18 =	\$	x \$ 36 =	\$ 432
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENTED			+ \$ 60 =	\$	+ \$ 20 =	\$
* If the difference in Col. 1 is less than zero, enter "0" in Col. 2.			TOTAL	\$	TOTAL	\$ 1042

- ☐ Please charge my Deposit Account No. 04-1644 in the amount of \$ _____.
☒ A check in the amount of \$ 1042.00 to cover the filing fee is enclosed.
☒ The Commissioner is authorized to charge payment of the following amounts associated with this communication or credit any overpayment to Deposit Account No. 04-1644:
☒ Additional filing fees under 37 CFR 1.16 or deficiencies in remittances therefor.
☒ Additional processing fees under 37 CFR 1.17 or deficiencies in remittances therefor.
☒ Any deficiency in any patent issue fee under 37 CFR 1.18 for which partial payment is made.

3. The enclosed utility patent application is related to _____

Date: August 25, 1990Attorney's Signature
Name and Reg. No.*Thomas Fitting*

Correspondence Address:

THOMAS FITTING, Reg. No. P-34,163

DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAIOW, LTD.
11300 Sorrento Valley Road, Suite 200
San Diego, California 92121
619/545-1555

EXHIBIT III

RB212458125

**POST OFFICE
TO ADDRESSEE**

**SEE REVERSE FOR SERVICE GUARANTEE
INSURANCE COVERAGE AND CLAIMS
THANK YOU FOR CHOOSING EXPRESS
MAIL SERVICE.**

ORIGIN Post Office: 92111 Zip Code: 92111 Receiving Office: 92111	Date In: 8/25/90 Time In: 12:01 P.M. Weight: 1.00 lb Dimensions: 10 x 6 x 6	Postage: 12.00 Return Receipt: YES Insurance: YES Signature: YES Postage & Fees: 12.00
ACCEPTANCE <input checked="" type="checkbox"/> First Class <input type="checkbox"/> Second Class <input type="checkbox"/> Third Class <input type="checkbox"/> Registered Mail <input type="checkbox"/> Certified Mail <input type="checkbox"/> Insured Mail		
Express Mail Service Corporate Account No. X921-884 Federal Agency Account No. _____ FROM: DRESSLER GOLDSMITH, 81 11900 SORRENTO VALLEY RD SAN DIEGO, CA 92121-1990		

Waiver of Signature and Indemnity (Domestic Only)

I wish delivery to be made without obtaining the signature of the addressee's agent (if in the judgment of the delivery employee article can be left in a secure location) and I understand the delivery employee will constitute valid proof of delivery.

SIGNED: _____

TO: PATENT AND TRADE MARK OFFICE
 Washington, DC 20530
 Attn: Hon. Commissioner

EXPRESS MAIL
NEXT DAY SERVICE

CUSTOMER RECEIPT



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

118

JAN 23 1991

Dressler, Goldsmith, Shore,
Sutker & Milnamow, Ltd.
11300 Sorrento Valley Road, Suite 200
San Diego, CA 92121

In re Application of :
Suzanne Zebedee, et al. :
Serial No. 07/573,643 : DECISION ON PETITION
Filed: August 27, 1990 :
For: NON-A, NON-B HEPATITIS VIRUS :
ANTIGEN, DIAGNOSTIC METHOD :
AND VACCINES :

The above-identified application has been forwarded to the Office of the Assistant Commissioner for Patents for consideration of the petition filed October 22, 1990, requesting that the application be accorded a filing date of August 25, 1990, instead of August 27, 1990.

Petitioners request the earlier filing date on the basis that the application was purportedly mailed to the Patent and Trademark Office (PTO) via "Express Mail" on August 25, 1990.

This application was deposited in U.S. Postal Service "Express Mail" on August 25, 1990, which was a Saturday, as evidenced by Express Mail customer receipt No. RB212458125. The application papers had a proper certificate of mailing by Express Mail. In accordance with the Patent and Trademark Office (PTO) procedures, however, the application papers were stamped in the Mail Room with the date of the next business day, Monday, August 27, 1990.

Applicants are now accorded the privilege of depositing applications by Express Mail and having the date of deposit considered the date of receipt in the PTO for filing date purposes. However, 37 CFR 1.10(a) and (c) specifically state that the date of deposit in Express Mail will not be accorded the papers if that date is a Saturday, Sunday or federal holiday within the District of Columbia. For example, 37 CFR 1.10(a) states:

"Any paper or fee to be filed in the Patent and Trademark Office can be filed utilizing the 'Express Mail Post Office to Addressee' service of the United States Postal Service and be considered as having been filed in the Office on the date the paper or fee is shown to have been deposited as 'Express Mail' with the United States Postal Service unless the date of deposit is a Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 CFR 1.6(a)."

Serial No. 07/573,643

Page 2

Accordingly, since August 25, 1990, was a Saturday, the above-identified application was properly accorded a filing date of August 27, 1990.

The petition is denied.

Receipt is acknowledged of the declarations, additional claim and surcharge fees filed on October 29, 1990, in response to the Notice To File Missing Parts of Application-Filing Date Granted mailed October 2, 1990.

The application is being returned to Examining Group 180 for further processing with a filing date of August 27, 1990.

C.E. Van Horn

Charles E. Van Horn
Patent Policy and Programs Administrator
Office of the A/C for Patents

msm/rkg



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/573,643	08/27/90	ZEBEDEE	S PHA0025P

EXAMINER
WORTMAN, D

ART UNIT	PAPER NUMBER
1802	9

DATE MAILED: 12/17/91

DRESSLER, GOLDSMITH, SHORE,
SUTKER & MILNAMOW, LTD.
11300 SORRENTO VALLEY RD., STE. 200
SAN DIEGO, CA 92121

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ *Restriction/Election Only*

☐ This application has been examined. ☐ Responsive to communication filed on _____ ☐ This action is made final.

A shortened statutory period for response to this action is set to expire: _____ month(s), 30 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- | | |
|---|---|
| 1. <input type="checkbox"/> Notice of References Cited by Examiner, PTO-892. | 2. <input type="checkbox"/> Notice re Patent Drawing, PTO-948. |
| 3. <input type="checkbox"/> Notice of Art Cited by Applicant, PTO-1449. | 4. <input type="checkbox"/> Notice of Informal Patent Application, Form PTO-152 |
| 5. <input type="checkbox"/> Information on How to Effect Drawing Changes, PTO-1474. | 6. <input type="checkbox"/> _____ |

Part II SUMMARY OF ACTION

1. ☒ Claims 1-40 are pending in the application.

Of the above, claims _____ are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☐ Claims _____ are rejected.

5. ☐ Claims _____ are objected to.

6. ☒ Claims 1-40 are subject to restriction or election requirement.

7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8. ☐ Formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____ Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).

10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).

12. ☐ Acknowledgement is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received; ☐ not been received. ☐ been filed in parent application, serial no. _____, filed on _____.

13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☐ Other

EXAMINER'S ACTION

Restriction to one of the following inventions is required under 35 U.S. 121:

- I. Claims 1-12, drawn to DNA, classified in Class 536, subclass 27.
- II. Claims 13-22, drawn to proteins, classified in Class 530, subclass 330.
- III. Claims 28-37, drawn to immunoassay, classified in Class 435, subclass 5.
- IV. Claims 23-27, drawn to kits containing antibodies, classified in Class 435, subclass 5.
- V. Claims 38-40, drawn to vaccine and method of immunizing, classified in Class 424, subclass 89.

The inventions are distinct, each from the other because of the following reasons:

The products of Group I, II, and IV are separate, distinct products. The DNA of Group I has other uses than in the production of the product of Group II, e.g., it can be used as a probe in a hybridization assay. The product of Group II can be obtained from sources other than the invention of Group I, i.e., it can be synthesized chemically or it can be purified from natural sources.

The product of Group I is not required for the inventions of Group III, Group IV, or Group V.

Inventions II and III are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product (M.P.E.P. § 806.05(h)). In the instant case the product of Group II has uses other than the method of Group III, e.g., in affinity purification.

Inventions II and V are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product (M.P.E.P. § 806.05(h)). In the instant case the product of Group II has uses other than the method of Group V, e.g., in the method of Group III, or in affinity purification.

The method of Group III does not require the invention of Group IV. The invention of Group IV can be used for other than immunoassays, e.g. the antibodies can be used in affinity

Serial No. 573643
Art Unit 1802

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purification of viral proteins where the antigen would be useful as a standard.

5 The methods of Group III and Group V are separate, distinct methods, each requiring different method steps and different reagents.

10 Because these inventions are distinct for the reasons given above and have achieved a separate status in the art as shown by their different classifications, restriction for examination purposes as indicated is proper.

15 In addition to the restriction requirement, the following election of species requirement is applied:

This application contains claims directed to the following patentably distinct species of the claimed invention: DNA sequences and amino acid sequences.

20 Applicant is required under 35 U.S.C. § 121 to elect a single disclosed species for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, no claim is generic.

25 Applicant is advised that a response to this requirement must include an identification of the species that is elected consonant with this requirement, and a listing of all claims readable thereon, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic
30 is considered nonresponsive unless accompanied by an election.

35 Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which are written in dependent form or otherwise include all the limitations of an allowed generic claim as provided by 37 C.F.R. § 1.141. If claims are added after the election, applicant must indicate which are readable upon the elected species. M.P.E.P. § 809.02(a).

40 Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the
45 inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. § 103 of the other invention.

Serial No. 573643
Art Unit 1802

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Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform to the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 308-4227.

Any inquiry concerning this communication should be directed to Donna C. Wortman at telephone number (703) 308-3988.

Dcw
Donna C. Wortman, Ph.D.
December 16, 1991

Esther L. Keplinger
ESTHER L. KEPLINGER
SUPERVISORY PATENT EXAMINER
GROUP ART UNIT 1802



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee et al.

Serial No.: 07/573,643

Filed: August 27, 1990

For: NON-A, NON-B HEPATITIS VIRUS
ANTIGEN, DIAGNOSTIC METHODS AND
VACCINES

Examiner: D. Wortman

Group Art Unit: 1802

Attorney Docket No.:
PHA-0025P

ELECTION

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231



Sir:

In response to the Action requiring restriction mailed December 17, 1991, claim Group III (claims 28-37) is hereby elected without traverse.

The Action requested election of species for DNA sequences and amino acid sequences. Claims to neither DNA nor amino acid sequences have been elected. As a consequence, it is believed that no further election of species is required.


In the event that the request for election of an amino acid sequence recited in the Action was meant to refer to proteins that include particular sequences as are recited in elected claim 28, a protein including an amino acid residue sequence represented by the sequence shown in Figure 1 from residue 1 to residue 74 is elected. Each of the elected claims reads substantially or completely on that elected species.

Serial No. 07/573,643

-2-

It is requested that all further correspondence be addressed to the undersigned counsel at the address shown on this paper..

Respectfully submitted,

By 
Edward P. Gamson, Reg. No. 29,381

Dressler, Goldsmith, Shore,
Sutker & Milnamow, Ltd.
4700 Two Prudential Plaza
180 North Stetson Avenue
Chicago, Illinois 60601
312/616-5400

CERTIFICATE OF MAILING

I hereby certify that this communication is being deposited with the United States Postal Services as First Class Mail, postage prepaid, in an envelope addressed to: Hon. Commissioner of Patents and Trademarks, Washington, D.C. 20231 on January 15, 1992.





UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/573,643	08/27/90	ZEBEDEE	FHA0023F

WORTMAN, EXAMINER

DRESSLER, GOLDSMITH, SHORE,
SUTKER & MILNAMOW, LTD.
11300 SORRENTO VALLEY RD., STE. 200
SAN DIEGO, CA 92121

ART UNIT 1802 PAPER NUMBER

DATE MAILED: 04/15/92

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined. ☐ Responsive to communication filed on _____ ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- | | |
|---|---|
| 1. <input checked="" type="checkbox"/> Notice of References Cited by Examiner, PTO-892. | 2. <input checked="" type="checkbox"/> Notice re Patent Drawing, PTO-948. |
| 3. <input type="checkbox"/> Notice of Art Cited by Applicant, PTO-1449. | 4. <input type="checkbox"/> Notice of Informal Patent Application, Form PTO-152 |
| 5. <input type="checkbox"/> Information on How to Effect Drawing Changes, PTO-1474. | 6. <input type="checkbox"/> _____ |

Part II SUMMARY OF ACTION

1. ☒ Claims 1-40 are pending in the application.
Of the above, claims 1-27, 38-40 are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 28-37 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____ Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been: ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____ has been: ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received, ☐ not been received ☐ been filed in parent application; serial no. _____; filed on _____.
13. ☒ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other: _____

Serial No. 573643
Art Unit 1802

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Applicant's election without traverse of Group III, Claims 28-37 in Paper No. 10 is acknowledged, as is the election of amino acid sequence shown in Fig. 1 from residue 1 to residue 74. Group III has been examined and all the claimed sequences have been treated in this action.

Because of the lengthy specification in this application, it has not been checked to the extent necessary to determine the presence of all possible minor errors. Applicant's cooperation is therefore requested in promptly correcting any errors of which he or she may become aware in the specification or drawings.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to provide an adequate written description of the invention and failing to teach how to make and/or use the invention.

The specification is not enabling for a method of assaying a sample for the presence of antibodies against a NANBV structural antigen by mixing the sample with a NANBV protein that includes the sequences as recited. Such a protein would necessarily occur in the virion per se and Applicant has not shown how to perform such an assay with whole virions; e.g. Applicant has not shown how to isolate and purify entire virions.

Claims 28-37 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

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Art Unit 1802

-3-

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

5 A patent may not be obtained though the invention is not
identically disclosed or described as set forth in section
102 of this title, if the differences between the subject
matter sought to be patented and the prior art are such that
the subject matter as a whole would have been obvious at the
10 time the invention was made to a person having ordinary
skill in the art to which said subject matter pertains.
Patentability shall not be negated by the manner in which
the invention was made.

15 Subject matter developed by another person, which qualifies
as prior art only under subsection (f) or (g) of section 102
of this title, shall not preclude patentability under this
section where the subject matter and the claimed invention
were, at the time the invention was made, owned by the same
person or subject to an obligation of assignment to the same
person.

20 This application currently names joint inventors. In
considering patentability of the claims under 35 U.S.C. § 103,
the examiner presumes that the subject matter of the various
claims was commonly owned at the time any inventions covered
therein were made absent any evidence to the contrary. Applicant
25 is advised of the obligation under 37 C.F.R. § 1.56 to point out
the inventor and invention dates of each claim that was not
commonly owned at the time a later invention was made in order
for the examiner to consider the applicability of potential 35
U.S.C. § 102(f) or (g) prior art under 35 U.S.C. § 103.

30 Claims 28-37 are rejected under 35 U.S.C. § 103 as being
unpatentable over Kuo et al. in view of Vyas, Neurath, and
Sugahara et al. and further in view of Takeuchi et al. Kuo
teaches an assay for hepatitis C virus antibodies using hepatitis
C proteins but does not teach use of hepatitis C structural
protein. Vyas, Neurath, and Sugahara all show use of other
hepatitis virus structural proteins to detect antibodies against
the virus but do not teach hepatitis C virus. Takeuchi teaches
the nucleotide and amino acid sequences of hepatitis C structural


Serial No. 573643
Art Unit 1802


-4-

proteins. It would have been obvious to one of ordinary skill in the art to use the hepatitis C structural protein sequence of Takeuchi to produce the hepatitis virus structural proteins as in Vyas, Neurath, and Sugahara and to use them in the hepatitis C antibody assay of Kuo with reasonable expectation for success because Vyas, Neurath, and Sugahara all teach that viral structural proteins contain antigenic determinants that are useful for detecting antibodies in sera of infected patients. One would have expected to be successful assaying for hepatitis C using the procedures of Vyas, Neurath, and Sugahara which have been successful for other viruses. Since the structural proteins are on the surface of the virus, one would have expected antibodies to have been raised against these proteins.

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform to the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 308-4227.

Any inquiry concerning this communication should be directed to Examiner Donna C. Wortman at telephone number (703) 308-1032.


Donna C. Wortman, Ph.D.
April 13, 1992


ESTHER L. KEPLINGER
SUPERVISORY PATENT EXAMINER
GROUP ART UNIT 1802

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

FORM PTO-892 (REV. 3-78)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		SERIAL NO. <u>272275</u> <u>573643</u>	GROUP/ART UNIT <u>1802</u>	ATTACHMENT TO PAPER NUMBER <u>11</u>		
NOTICE OF REFERENCES CITED				APPLICANT(S) <u>Zebedee et al.</u>				
U.S. PATENT DOCUMENTS								
•		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE	
	A	<u>4415491</u>	<u>11/83</u>	<u>Vyas</u>	<u>436</u>	<u>820</u>		
	B	<u>4591552</u>	<u>5/86</u>	<u>Neurath</u>	<u>435</u>	<u>5</u>		
	C	<u>4839277</u>	<u>6/89</u>	<u>Sugahara et al.</u>	<u>435</u>	<u>5</u>		
	D							
	E							
	F							
	G							
	H							
	I							
	J							
	K							
FOREIGN PATENT DOCUMENTS								
•		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG. PP. SPEC.
	L							
	M							
	N							
	O							
	P							
	Q							
OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)								
	R	<u>Kuo et al., Science 244: 362-364. 1989.</u>						
	S	<u>Tateuchi et al., Nucl. Acid Res. 18: 4626. 1990.</u>						
	T							
	U							
EXAMINER <u>Rene Clout</u>		DATE <u>4/3/90</u>						
* A copy of this reference is not being furnished with this office action. (See Manual of Patent Examining Procedure, section 707.05 (a).)								

ATTACHMENT TO PAPER NUMBER	11
APPLICATION NUMBER	573643

NOTICE OF DRAFTSMAN'S PATENT DRAWING REVIEW

THE PTO DRAFTSMEN REVIEW ALL ORIGINALLY FILED DRAWINGS REGARDLESS OF WHETHER THEY WERE DESIGNATED AS INFORMAL OR FORMAL.

The drawings filed 8/27/90

- A. ☐ are approved.
- B. ☒ are objected to under 37 CFR 1.84 for the reason(s) checked below. The examiner will require submission of new, corrected drawings at the appropriate time. Corrected drawings must be submitted according to the instructions listed on the back of this Notice.

1. Paper and Ink. 37 CFR 1.84(a)

☐ Sheet(s) _____ Poor.

2. Size of Sheet and Margins. 37 CFR 1.84(b)

Acceptable Paper Sizes and Margins

Margin	Paper Size		
	8 1/2 by 14 inches	8 1/2 by 13 inches	DIN size A4 21 by 29.7 cm.
Top	2 inches	1 inch	2.5 cm.
Left	1/4 inch	1/4 inch	2.5 cm.
Right	1/4 inch	1/4 inch	1.5 cm.
Bottom	1/4 inch	1/4 inch	1.0 cm.

☒ Proper Size Paper Required.
All Sheets Must be Same Size.
Sheet(s) 4

☐ Proper Margins Required.
Sheet(s) _____

☐ TOP ☐ RIGHT
☐ LEFT ☐ BOTTOM

3. Character of Lines. 37 CFR 1.84(c)

☐ Lines Pale or Rough and Blurred.
Fig(s) _____

☐ Solid Black Shading Not Allowed.
Fig(s) _____

4. Hatching and Shading. 37 CFR 1.84(d)

☐ Shade Lines are Required.
Fig(s) _____

☐ Criss-Cross Hatching Not Allowed.
Fig(s) _____

☐ Double Line Hatching Not Allowed.
Fig(s) _____

☐ Parts in Section Must be Hatched.
Fig(s) _____

5. Reference Characters. 37 CFR 1.84(f)

☒ Reference Characters Poor or Incorrectly Sized.
Fig(s) 1-2 (1/2" tall)

☐ Reference Characters Placed Incorrectly.
Fig(s) _____

6. Views. 37 CFR 1.84(i) & (j)

☐ Figures Must be Numbered Properly.

☐ Figures Must Not be Connected.
Fig(s) _____

7. ☐ Photographs Not Approved.

8. ☐ Other.

Telephone inquiries concerning this review should be directed to the Chief Draftsman at telephone number (703) 557-6404.

Wm D S
Reviewing Draftsman

11/27/90
Date



UNITED STATES PATENT AND TRADEMARK OFFICE

MW
4-27-92
#12

Applicant: Zebedee et al.) PATENT APPLICATION
Serial No.: 07/573,643) Examiner: D. Wortman
Filed: August 25, 1990) Group Art Unit: 1802
For: NON-A, NON-B, HEPATITIS)
VIRUS ANTIGEN, DIAGNOSTIC)
METHODS AND VACCINES)

INFORMATION DISCLOSURE STATEMENT

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Pursuant to 37 C.F.R. §1.97, a list of documents is disclosed on the attached form PTO-1449 that may be material to the examination of this application. The subject application is one of three related applications referred to herein as the "grandparent", "parent", and "child" applications. The serial numbers and filing dates of those applications are 07/573,643 filed on August 25, 1990 (the subject and grandparent application), 07/616,369 filed on November 21, 1990 (the parent application and a C-I-P of the grandparent application), and 07/748,564 filed on August 21, 1991 (the child application and a C-I-P application of the parent application).

Listed documents A and D-N on the attached form PTO-1449 are cited and discussed in all three applications.

Listed documents O-Z are recited and discussed only in the child application.

Listed documents AA-AG are included on the list as general background art related to the work of some of the present inventors on non-A/non-B hepatitis viruses.

Listed documents B, C and AH were cited in the International Search Report for PCT Application PCT/US91/06037, which application corresponds to the child application. A copy of that International Search Report is enclosed for the Examiner's convenience. A copy of each of the documents is enclosed herewith for the Examiner's consideration.

No inferences should be drawn that the attached list represents a comprehensive investigation, or that any material disclosed is equivalent to the subject invention. In addition, none of the documents that have publication dates prior to the priority date of the above application anticipate the invention in this application.

The cited documents disclose numerous specific features. There has been no attempt to list each and every feature disclosed by each document. The Examiner is requested to review the documents and determine the extent of the materiality of the document disclosures with respect to the present invention.

The recitation of any art or document herein is not to be construed as an admission that the art or document disclosure is necessarily within the invention field of endeavor, that the art or document disclosure is necessarily prior in time to a particular date which may be relevant to the instant patent application, and/or that the art or document disclosure is otherwise necessarily prior art as defined by the patent law with respect to the instant invention and application.

Also, there is reserved the right to later set forth how the instant invention is distinguished over the disclosure of any document or other art, including the disclosures of the art and documents recited herein, that may be cited by the Examiner in rejecting a claim in the instant patent application.

07/573,643

-3-

The recitation herein of the art and documents is not to be construed as an assertion that more pertinent art could not possibly be in existence.

Respectfully submitted,

By Edward P. Gamson
Edward P. Gamson, Reg. No. 29,381

Enclosures

1. Form PTO-1449;
2. A copy of the International Search Report for PCT Application PCT/US91/06037, which corresponds to U.S. Patent Application S/N 07/748,564 and;
3. Copies of the cited art

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CERTIFICATION OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Hon. Commissioner of Patents and Trademarks, Washington, D.C. 20231 on April 10, 1992.

Edward P. Gamson
Edward P. Gamson

Serial No.
07/573,643

INFORMATION DISCLOSURE CITATION
(Use several sheets if necessary)

Commercial Attorney Docket N
rk Office R PHA-0025
APR 11 1992
Applicant
Zebedee et al.
Filing Date
August 25, 1990

Group
1802

U.S. PATENT DOCUMENTS

[illegible]

FOREIGN PATENT DOCUMENTS

[illegible]

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

Down	D	Choo et al, <u>Science</u> , <u>244</u> , 359-362 (1989)
	E	Okamoto et al, <u>Japan J. Exp. Med.</u> , <u>60</u> , 163-177 (1990)
↓	F	Miller et al., <u>PNAS</u> <u>87</u> , 2057-2061 (1990)
	G	Kuo et al, <u>Science</u> <u>244</u>, 362-366 (1989) already cited in P to 892
Down	H	Alter et al, <u>NEJM</u> <u>321</u> , 1538-39 (1989)

Examiner

Date Considered,

*Examiner: Initial if citation considered, whether or not citation is in conformance with MPEP § 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Form PTO-1449 (Rev. 8-88)		U.S. Department of Commerce Patent and Trademark Office		Attorney Docket No. PHA-0025	Serial No. 07/573,643
INFORMATION DISCLOSURE CITATION (Use several sheets if necessary)				Applicant Zebedee et al.	
				Filing Date August 25, 1990	
Group 1802					
OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)					
	I	Alter et al, <u>NEJM</u> , <u>321</u> , 1494-1500 (1989)			
	J	Weiner et al, <u>Lancet</u> , <u>335</u> , 1-3 (1990)			
	K	McFarlane et al, <u>Lancet</u> , <u>335</u> , 754-757 (1990)			
	L	Grey et al, <u>Lancet</u> , <u>335</u> , 609-610 (1990)			
	N	Houghton et al, <u>Int. K. Prot Res</u> , <u>16</u> , 311-320 (1980)			
	O	Choo et al, <u>PNAS</u> , <u>88</u> 2451-2455 (1991)			
	P	Takamizawa et al., <u>J. Virol.</u> , <u>65</u> , 1105-1113 (1991)			
	Q	Kato et al, <u>PNAS</u> , <u>87</u> , 9524-9528 (1990)			
	R	Takeuchi et al., <u>Nucleic Acids Res.</u>, <u>18</u>, 4626-4630 (1990) cited in PTO 892			
	S	Ogata et al, <u>PNAS</u> , <u>88</u> , 3392-3396 (1991)			
	T	Hen et al., <u>PNAS</u> , <u>88</u> , 1711-1715 (1991)			
	U	Meyer et al., <u>Virol</u> , <u>171</u> , 555-567 (1989)			
	V	Collett et al., <u>Virol</u> , <u>165</u> , 191-199 (1988)			
	W	Brinton et al., <u>Virol</u> , <u>162</u> , 290-299 (1988)			
	X	Inchauspe et al, <u>PNAS</u> , <u>88</u> , 10292-10296 (1991)			
	Y	Wiener et al, <u>Virol</u> , <u>180</u> , 842-848 (1991)			
	Z	Hahn et al, <u>Virol</u> , <u>162</u> , 167-180 (1988)			
	AA	Prince et al, <u>Lancet</u> , 2:241 (1974)			
	AB	Prince et al., "Posttransfusion Viral Hepatitis Caused by an Agent or Agents Other Than Hepatitis B Virus or Hepatitis A Virus. Impact On Efficiency of Present Screening Methods." in <u>Transmissible Disease & Blood Transfusion</u> , Tibor et al. eds., Grune & Stratton, Inc., pp. 129-140 (1975)			
	AC	Prince et al., "Non-A/Non-B Hepatitis: Identification of a virus-specific antigen and antibody. A preliminary report" in <u>Viral Hepatitis</u> , Vyas et al., eds., Franklin Institute Press, Philadelphia, Pa. pp. 633-640 (1978).			
	AD	Prince et al., "Non-A, Non-B Hepatitis: Reproduction of disease in chimpanzees and identification of virus specific antigen and antibody" in <u>Transplantation and Clinical Immunology</u> , Volume X, Touraine et al., eds., Excerpta Medica, Amsterdam, pp. 8-17 (1979).			
	AE	Prince, A.M., <u>Lancet</u> , May 22, 1982.			
Examiner		Date Considered			
Donna C. Worthington		9/22/90			
*Examiner: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.					



Atty. Docket PHA0025

THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee et al.

PATENT APPLICATION

Serial No.: 07/573,643

Examiner: D. Wortman

Filed: August 27, 1990

Group Art Unit: 1802

For: NON-A, NON-B, HEPATITIS
VIRUS ANTIGEN, DIAGNOSTIC
METHODS AND VACCINES

13/a
D. Wortman
7/30/92
RECEIVED

AMENDMENT UNDER 37 C.F.R. § 1.115

JUL 27 1992

GROUP 1800

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

This Amendment is being filed in response to the April 15, 1992 Office Action (paper no. 11) issued in connection with the above-captioned patent application.

IN THE CLAIMS:

Please cancel claims 1-27 and 38-40 without prejudice to their being represented in a divisional application.

Please amend claims 28, 29 and 30 as follows:

B1
28. (Amended) A method of assaying a body fluid sample for the presence of antibodies against [a] NANBV [structural antigen], which method comprises:

a) forming an immunoreaction admixture by admixing said body fluid sample with a recombinant NANBV structural protein or synthetic polypeptide portion thereof, said recombinant protein or polypeptide including an amino acid residue sequence represented by the sequence shown in Figure 1 from residue 1 to residue 74, from residue 69 to residue 120, or from residue 121 to residue 176;

b) maintaining said immunoreaction admixture for a time period sufficient for any of said antibodies present to

immunoreact with said recombinant NANBV structural protein or synthetic polypeptide to form an immunoreaction product;

and

c) detecting the presence of any of said immunoreaction product from and thereby the presence of said antibodies.

29. (Amended) The method of claim 28 wherein said recombinant NANBV structural protein or polypeptide has an amino acid residue sequence shown in Figure 2 from residue 1 to residue 316.

30. (Amended) The method of claim 28 wherein said recombinant NANBV structural protein or polypeptide is affixed to a solid matrix.

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the discussion that follows is respectfully requested.

In claims 1-28 and 38-40 have been cancelled in view of the restriction requirement and without prejudice to their being presented again in a divisional application. Claims 28-30 have been amended. Claims 28-37 are before the Examiner.

I. THE AMENDMENTS

Support for the addition of the words "recombinant" and "synthetic polypeptide" to claims 28-30 can be found in the specification at least at page 20, line 8 to page 22, line 25. Those pages of the specification set forth methods for making a recombinant structural protein and synthetic polypeptide of the present invention.

Exemplary use of a method of the invention can be found at least at page 68, line 18 through page 73, line 5. Those pages of the specification exemplify the use of a recombinant NANBV structural protein in an assay for the detection of antibodies against NANBV.

II. Rejection Under 35 U.S.C. § 112, First And Second Paragraphs

The Action has objected to the specification under 35 U.S.C., Section 112, first paragraph as failing to provide an adequate written description of the invention and as failing to teach how to make and use the invention. The Action has rejected claims 28 and 37 under 35 U.S.C. §112, first paragraph for the reason set forth in the objection to the specification. In particular, the Action asserts that the specification is not enabling for a method of assaying a body fluid sample for the presence of antibody against a NANBV structural antigen by mixing the sample with a NANBV structural protein because such a protein would necessary occur in the virion and that Applicant has not shown how to perform such an assay.

In view of the clarifying amendments to claim 28, this rejection should be moot. Thus, a recombinant protein or a synthetic polypeptide portion thereof would not be present in a virion.

III. Rejection Under 35 U.S.C. § 103

The Action has rejected claims 28-37 under 35 U.S.C §103 as being unpatentable over Kuo et al., (hereinafter referred to as "Kuo") in view of Vyas, Neurath, and Sugahara et al. (hereinafter referred to as "Sugahara") and further in view of Takeuchi et al. (hereinafter referred to "Takeuchi"). The Action characterizes:

- 1) Kuo as teaching assays for hepatitis C virus antibodies using hepatitis C proteins, but not using hepatitis C structural proteins;
- 2) Vyas, Neurath and Sugahara as teaching the use of hepatitis B virus structural proteins to detect antibodies against the virus, but not teaching use of hepatitis C virus proteins; and
- 3) Takeuchi as teaching the nucleotide and amino acid sequence of hepatitis C structural proteins.

In view of that characterization, the Action concludes that it would have been obvious to one of ordinary skill in the art to

use the hepatitis C structural protein sequence of Takeuchi to produce hepatitis viral structural proteins as in Vyas, Neurath and Sugahara and to use those proteins in the hepatitis C antibody assay of Kuo with reasonable expectation of success. In support of that conclusion, the Action further asserts that because Vyas, Neurath and Sugahara all teach that viral structural proteins contain antigenic determinants that are useful in detecting antibodies in sera of infected patients, one would have expected to be successful in assaying for hepatitis C using those proteins. The Action still further states that because the structural proteins are on the surface of the virus, one would have expected antibodies to have been raised against those proteins.

That rejection by the Action is respectfully traversed on the bases that (1) the Action has credited the cited art with teachings not contained therein, (2) the art is not properly combineable and (3) even if combined, those combined teachings fall short of describing the present invention.

A. First Basis

First, the Action characterizes Kuo as teaching assays for antibodies against hepatitis C virus using hepatitis C proteins, but not structural proteins. It is respectfully submitted that the teaching of Kuo is more narrow than indicated by the Action.

Kuo actually teaches an assay for antibodies against hepatitis C virus using a single fusion protein comprising 363 viral amino acid residues of non-structural protein. As noted in the large paragraph at page 3 of the specification, the Kuo construct is a fusion protein containing products of two non-structural protein genes.

Second, the Action credits Vyas, Neurath and Sugahara with teaching that hepatitis B structural proteins have use in an assay for detecting antibodies against the virus. It is submitted that the Vyas, Neurath and Sugahara teachings, all of

which relate to hepatitis B viral surface and core proteins, are not relevant here, and that those teachings should be withdrawn.

As is pointed out in the specification at page 2, beginning at line 12, the NANBV genome is comprised of a single, plus strand of RNA that encodes a single polyprotein. The hepatitis B virus (HBV) genome is double-stranded circular DNA. Hepatitis B virus belongs to a novel class of enveloped hepatropic DNA viruses, the hepadnavirus family (see Exhibit A, attached hereto). Document F of the recently filed Information Disclosure Statement points out that NANBV has similarities to the animal pestiviruses and plant carmovirus and polyvirus.

Thus, except for the facts that both are viruses and both NANBV and HBV infect the liver and cause inflammation (hepatitis), the two viruses have little in common. That being the case, it is submitted to be improper to draw any conclusion as to any other similarity of properties between the two viruses, let alone antigenicity or immunogenicity of proteins from the two viruses.

The Takeuchi teaching provides a naked DNA sequence and putative amino acid residue sequence in which the core and envelope regions of the fusion protein are putatively assigned. No mention is made of any encoded region that might have use in an assay for anti-HCV antibodies.

In view of the above, it is respectfully submitted that the Action has credited the cited art with teachings not disclosed therein.

B. Second Basis

To establish a prima facie case of obviousness based on a combination of teachings, 1) the teachings themselves must suggest the combination or 2) there must be a compelling motivation to combine the teachings, which motivation is based on sound scientific principles. Ex parte Kranz, 19 USPQ2d 1218 (Bd. Pat. App. Inter. 1991).

1. Art-based suggestion to combine

As set forth above, Kuo teaches an assay for antibodies against hepatitis C virus using a single fusion protein comprising 363 viral amino acid residues. Kuo neither mentions nor suggests that structural proteins or portions thereof can be used in his assay. In addition, Kuo fails to even mention any other virus, let alone another hepatitis virus. Kuo, therefore, cannot be viewed as suggesting the combination proposed by the Action.

The suggestion for combination is not provided by Vyas, Neurath or Sugahara. None of that cited art discloses peptides or assays for detecting antibodies against a virus other than HBV, a virus already shown to be quite different. Further, none of that art relates to hepatitis C. Still further, Vyas and Neurath teach use of peptides to a surface structural protein, HbsAg, whereas the sequences claimed here relate to the capsid. It is submitted that no properly suggestive inference can be drawn from results with the surface protein to results with the capsid protein of an entirely different virus. Reliance on Vyas and Neurath should therefore be withdrawn.

Although Takeuchi discloses the nucleic acid and derived amino acid residue sequences of HCV structural proteins, Takeuchi does not teach the location or identity of any antigenic determinants that might have use in designing an assay for detection of antibodies against HCV as claimed herein. In view of the above, it is respectfully submitted that the cited art cannot provide the requisite motivation for combination as proposed by the Action, and this rejection should be withdrawn.

2. Compelling motivation based on sound scientific principles

There is no compelling motivation based on sound scientific principles to combine the art cited by the Action. First, the teachings of Vyas, Neurath and Sugahara relate to hepatitis B virus. Those teachings have application to the teachings of Kuo and Takeuchi (related to hepatitis C virus) only if there is a

known relationship between those viruses. The Action provides no evidence to support such a relationship.

To the contrary, the art relied upon by the Action and that provided earlier shows that hepatitis B and C differ substantially in structure. Further, the art points out that major differences between those viruses occur in genomic coding and protein expression of antigens, the very portions of the virus giving rise to the Action's reliance.

Vyas and Neurath disclose peptides associated with the hepatitis B surface antigen. That antigen is known to be expressed on the outer covering of the hepatitis B virus (See the article from Laboratory Investigation enclosed herewith as Exhibit A).

Notably absent from the teachings of Kuo is any mention whatsoever of a surface antigen encoded by a hepatitis C genome or expressed by that virus. Even if there were such a teaching, it would not be relevant as the sequences claimed herein are from the capsid.

It can thus be seen that none of the cited art provides any guidance on what part, if any, of the hepatitis C genome or proteins expressed therefrom might have any use. Even if one of ordinary skill in the art were motivated to combine the cited art, such an artisan would be unable to do so. There is simply no teaching anywhere in the art of record to make or use proteinaceous material having the amino acid residue sequence claimed.

Second, the Action-cited art combination also seems to be predicated on the assumption that, because short peptides that mimic portions of structural antigens have been shown to immunoreact with antibodies against intact antigens, a worker skilled in the art would have a reasonable expectation of success in there substantially always being such interactions. Not only does the Action fail to provide any evidence in support of that assumption, but there is evidence to support exactly the contrary.

Enclosed herewith as Exhibit B is an article from Science discussing antibody-protein interaction. The Examiner's attention is respectfully directed to the second full paragraph of page 662 of that article that begins near the bottom of the page. That paragraph points out that although short peptides can be used to prepare anti-protein antibodies, it is a rare event for a short peptide to be antigenic (i.e., immunoreact with antibodies against the intact protein). In view of that teaching, one of ordinary skill in the art would not be motivated to look for small linear peptides as a means for detecting antibodies against intact viruses.

It is therefore respectfully submitted that there is no compelling motivation based on sound scientific principles to combine the cited art in the manner proposed by the Action. When taken together with the absence of any art-based motivation for such a combination, it is further respectfully submitted that the art relied upon by the Action is not properly combineable, and this rejection should be withdrawn.

C. Third Basis

Even assuming arguendo, however, that the Action-proposed combination of art were proper, such a combination falls short of describing the present invention.

The present invention relates to an assay for antibodies against NANBV using a NANBV structural protein that includes small amino acid residue sequences (up to about 65 amino acid residues) from the putative capsid antigen of NANBV (residues 1-74, 69-120 or 121-176 of Figure 1. The structural protein can be a fusion protein (claim 29) that contains a short residue sequence.

The assay of Kuo, as admitted by the Action, does not employ a NANBV structural protein. The teachings of Vyas, Neurath and Sugahara cannot provide that structural protein for at least two reasons. First, the peptides disclosed in that art are derived from HBV, not NANBV. Second, there is no basis in the record to

conclude that all viral structural proteins are immunogenic or antigenic, nor that the particular regions of NANBV here claimed would be antigenic. Because Takeuchi does not even discuss antigenic determinants, his disclosure adds nothing to Kuo to provide the worker of ordinary skill with the required reasonable expectation of success. In Re O'Farrell, 7 USPQ 1673, 1681 (Fed. Cir. 1988).

In light of the reasons set forth above, it is respectfully requested that the rejection of claims 28-37 under 35 U.S.C §103 be withdrawn.

D. Enhanced Results

The Examiner's attention is also invited to the results obtained using a claimed method that are provided at page 68, line 18 through page 73, line 5. The results shown in head-to-head comparisons of the Kuo construct in a commercial Kit (Anti HCV) and an assay of this invention (Anti Cap-N, Tables 2-6) illustrate that an assay using a claimed method provided enhanced results as compared to the Kuo construct. Thus, an assay of the present invention was able to detect HCV infection one or more months earlier than could an assay using the Kuo construct. Those results were thoroughly unpredictable, and illustrate the non-obviousness of this invention.

Summary

Claims 1-27 and 38-40 have been cancelled and claims 28-30 amended. Each of the bases for objection or rejections have been dealt with and make moot or otherwise overcome.

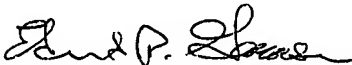
In view of the amendments to the claims and for the foregoing reasons, it is respectfully submitted that the claims

07/573,643

-10-

now stand in a condition of allowance. Early notification to that effect is respectfully requested.

Respectfully submitted,

By 
Edward P. Gamson, Reg. No. 29,381

Enclosures

1. Exhibits A-B and;
2. Form PTO-1449

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07/573,643 15



-11-

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JUL 27 1992

GROUP 1800

CERTIFICATION OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Hon. Commissioner of Patents and Trademarks, Washington, D.C. 20231 on July 15, 1992.

Edward P. Gamson

GROUP 1800

Sheet 1 of 1

Serial No. 071573

Group 1802

U.S. PATENT DOCUMENTS

FOREIGN PATENT DOCUMENTS

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

Sutcliffe et al., Science, 219(4585), 660:666 (1983)

Date Considered

Initial if citation considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.



**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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07/573,643 08/27/90 ZEBEDEE

S. PHA0025P

EXAMINER

WORTMAN, D

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ART UNIT PAPER NUMBER

1802

DATE MAILED: 10/05/92

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined. ☒ Responsive to communication filed on 7/20/92 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 2 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☒ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form:PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐

Part II SUMMARY OF ACTION

1. ☒ Claims 28-37 are pending in the application.
Of the above, claims _____ are withdrawn from consideration.
2. ☒ Claims 1-27 and 38-40 have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 28-37 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____ Under 37 C.F.R. 1.84 these drawings are ☐ acceptable ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved ☐ disapproved (see explanation).
12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received
☐ been filed in parent application, serial no. _____; filed on _____
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

Claims 28-37 are currently under examination, Claims 1-27 and 38-40 having been cancelled in Paper No. 13. Claims 28, 29, and 30 have been amended.

5 Claims 28-37 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 28 is indefinite because it is unclear whether "including an amino acid sequence" is intended to
10 encompass only sequences with the same specific start and end points as recited or every protein or polypeptide with any amino acid sequence in common with those specifically recited.

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

5 A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

10 (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

15 (c) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

20 The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

25 A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.
30 Patentability shall not be negated by the manner in which the invention was made.

Serial No. 573643
Art Unit 1802

-3-

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. § 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 C.F.R. § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. § 102(f) or (g) prior art under 35 U.S.C. § 103.

Claims 28, 30, 31, 33, and 36 are rejected under 35 U.S.C. § 102(e) as anticipated by or, in the alternative, under 35 U.S.C. § 103 as obvious over the patent to Wang. Wang teaches assaying sera for antibodies against HCV (NANBV) using solid phase coated with synthetic peptides that include amino acid residue sequences as instantly claimed (see Wang, Example 14 and Table 7, especially peptides VIIIE, IXD, and IXE). It is noted that "including" as recited in Claim 28 encompasses any common amino acid sequence. Even if the instant sequences were recited more narrowly, they would have been obvious over Wang because there are only slight differences between them and the core sequences taught by Wang.

Claims 32, 34, 35, and 37 are rejected under 35 U.S.C. § 103 as being unpatentable over Wang. Wang teaches the assay for HCV antibodies as discussed but does not teach protein A for binding to immunoglobulin nor specifically describe lanthanide chelate,

biotin, or radioactive isotopes as labels. These variations are well known in the art and it would have been obvious to one of ordinary skill in the art to substitute them for the anti-human immunoglobulin antibody and the enzyme label of Wang with
5 reasonable expectation for success because they are well known and conventionally used in immunoassays.

Claim 29 is rejected under 35 U.S.C. § 103 as being unpatentable over Wang in view of Kuo et al. Wang teaches the HCV peptide sequences discussed above but does not teach
10 producing them recombinantly. Kuo teaches production of an HCV recombinant fusion protein. It would have been obvious to one of ordinary skill in the art to produce the HCV peptide of Wang recombinantly as taught by Kuo in order to gain the advantages of producing peptides by recombinant means, e.g., to obtain a
15 stable, plentiful supply of peptides that are free of contamination with other HCV antigens.

Because this action contains new grounds of rejection, it is made non-final. Any resulting inconvenience is regretted.

Applicant's arguments with respect to claims 28-37 in Paper
20 No. 13 have been considered but are deemed to be moot in view of the new grounds of rejection.

Papers related to this application may be submitted to Group
180 by facsimile transmission. Papers should be faxed to Group
180 via the PTO Fax Center located in Crystal Mall 1. The faxing
25 of such papers must conform to the notice published in the

Serial No. 573643
Art Unit 1802

-5-

Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax
Center number is (703) 308-4227.

Any inquiry concerning this communication should be directed
to Examiner Donna C. Wortman at telephone number (703) 308-1032.

5

Dcw
Donna C. Wortman, Ph.D.
September 28, 1992

Esther Kepplinger
ESTHER L. KEPPLINGER
SUPERVISORY PATENT EXAMINER
GROUP ART UNIT 182-1802

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

272275

FORM PTO-892 (REV. 3-78)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO. 573643	GROUP/ART UNIT 1802	ATTACHMENT TO PAPER NUMBER 14
NOTICE OF REFERENCES CITED		APPLICANT(S) Zebedee et al.		

U.S. PATENT DOCUMENTS

	DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
A	5106726	4-1992	Wang	435	5	7-1990
B						
C						
D						
E						
F						
G						
H						
I						
J						
K						

FOREIGN PATENT DOCUMENTS

	DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG.	PP. SPEC.
L								
M								
N								
O								
P								
Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

R	
S	
T	
U	

EXAMINER Verna C. Woot	DATE 9/25/92
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* A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#15
R.K.
2-12-93

Applicant: Zebedee et al.)
Serial No.: 07/573,643) Attorney Docket
Filed: August 27, 1990) PHA 0025P
For: NON-A, NON-B, HEPATITIS VIRUS)
ANTIGEN, DIAGNOSTIC METHODS AND)
VACCINES)
Examiner: D. Wortman)

PETITION UNDER 37 C.F.R. §1.17

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

A one-month extension of time to respond to the Office
Action mailed October 5, 1992 is respectfully requested.

There is submitted herewith the following:

1. Response;
2. Declaration of Alfred M. Prince, M.D.;
3. Form PTO-1449;
4. Documents BA through BE;
5. Exhibit I; and
6. Check No. 19286 in the amount of the required fee
of \$110.00 for a one-month extension of time (a response to the
Office Action was due on January 5, 1993).

The Commissioner is hereby authorized to charge payment
of any additional fees under 37 C.F.R. §1.17 to cover the cost of
the extension or credit any overpayment to Deposit Account No.
04-1644. A duplicate copy of this paper is enclosed.

Respectfully submitted,

CS13061 02/16/93 07573643

04-1644 140 115 110.0000

By Edward P. Gamson
Edward P. Gamson, Reg. No. 29,381

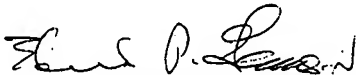
Serial No. 07/573,643

-2-

DRESSLER, GOLDSMITH, SHORE,
SUTKER & MILNAMOW, LTD.
4700 Two Prudential Plaza
180 North Stetson Avenue
Chicago, Illinois 60601
312/616-5400

CERTIFICATE OF MAILING

I hereby certify that this Petition, in duplicate, together with the aforementioned enclosures is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Hon. Commissioner of Patents and Trademarks, Washington, D.C. 20231, on January 28, 1993.



Edward P. Gamson

DRESSLER, GOLDSMITH, SHORE
SUTKER AND MILNAMOW, LTD.

180 N. STETSON AVENUE
CHICAGO, ILL. 60601

19286

2-91/710

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FOR

J. D. Meyer

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THE UNITED STATES PATENT OFFICE IS REQUESTED TO IMPRESS
ITS STAMP ON THIS CARD AND PLACE SAME IN THE OUT-GOING
MAIL TO SHOW THE FOLLOWING PAPERS HAVE BEEN RECEIVED.

PHA-0025P

EPG:ja

Applicants: Zebedee et al.
Serial No.: 07/573,643
Group: 1802

Enclosed: AMENDMENT, certified mailed January 28, 1993,
together with Declaration of Alfred M. Prince,
certified mailed January 28, 1993; Form PTO-1449;
Documents BA through BE; Exhibit I; Petition for
a one-month extension of time, in duplicate,
certified mailed January 28, 1993; and Check
No. 19286 in the amount of \$110.00 for the
one-month extension of time.

187
1/16/92
041
16/18
2-12-92
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

94
2/17/93
Applicant: Zebedee et al.)
Serial No.: 07/573,643) Attorney Docket
Filed: August 27, 1990) PHA 0025P
For: NON-A, NON-B, HEPATITIS VIRUS)
ANTIGEN, DIAGNOSTIC METHODS AND)
VACCINES)
Examiner: D. Wortman)

RECEIVED

FEB 1 - 1993

GROUP 1800

AMENDMENT UNDER 37 C.F.R. §1.115

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

In response to the Official Action dated October 5, 1992, for which a Petition for an extension of time and its required fee are enclosed, please amend the above-identified application as follows.

IN THE SPECIFICATION

At page 63, line 29, please delete "amin-terminal", and replace it with --amino-terminal--.

At page 64, line 6, please delete "correspondingto", and replace it with --corresponding to--.

IN THE CLAIMS

Please amend claims 28, 29 and 30 as follows:

28. (Twice Amended) A method of assaying a body fluid sample for the presence of antibodies against NANBV, which method comprises:

a) forming [a] an immunoreaction admixture by admixing said body fluid sample with a recombinant NANBV structural protein or [synthetic polypeptide] (portion) thereof, said recombinant protein or [polypeptide] portion (including) an amino acid residue sequence represented by the sequence shown in

figure 1 from residue 1 to residue 74[, from residue 69 to residue 120, or from residue 121 to residue 176] or from residue 1 to residue 120;

b) maintaining said immunoreaction admixture for a time period sufficient for any of said antibodies present to immunoreact with said recombinant NANBV structural protein or [synthetic polypeptide] portion to form an immunoreaction product;

and

c) detecting the presence of any of said immunoreaction product from and thereby the presence of said antibodies.

29. (Twice Amended) The method of claim 28 wherein said recombinant NANBV structural protein or [polypeptide] portion has an amino acid residue sequence shown in Figure 2 from residue 1 to residue 316.

30. (Twice Amended) The method of claim 28 wherein said recombinant NANBV structural protein or [polypeptide] portion is affixed to a solid matrix.

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the discussion that follows is respectfully requested.

Claims 28, 29 and 30 have been amended as discussed below. Claims 28-37 are before the Examiner.

I. The Amendments

Typographical errors have been corrected at page 63, line 29 and page 64, line 6.

Claims 28-30 have been amended to recite only recombinantly produced particular (NANBV proteins or portions

thereof, with references to "synthetic" polypeptide portions being deleted. The use of such recombinant proteins is discussed throughout the specification and is exemplified in the working examples, as will be discussed hereinafter.

Claim 28 has also been amended to recite the sequence complete recombinant protein. That protein is discussed in several places in the specification. One exemplary location is in the paragraph at page 12, lines 3-9, as well as at page 23, lines 14-18.

It is thus seen that no new matter has been added.

II. THE ACTION

A. Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 28-37 were rejected as allegedly being indefinite for their inclusion of the language "including an amino acid [residue] sequence". The Action stated that

"it is unclear whether [the quoted phrase] is intended to encompass only sequences with the same specific start and points as recited or every protein or polypeptide with any amino acid [residue] sequence in common with those specifically recited."

This rejection is respectfully traversed.

The use of the term "including" has been blessed by the courts in cases such as In re Bertsch, 56 USPQ 380 (CCPA 1942) and Gould v. Mossinghoff, 215 USPQ 310; rev. on other grounds 219 USPQ 393 (CCPA 1983). The words "including" and "comprising" were held to be synonymous in Bertsch, and both were held to be "inclusive" in Gould. In addition, Aisenberg, I.M., Attorney's Dictionary of Patent Claims, Matthew Bender & Co. (1992), pp. I-10.1 through I-13 (copy enclosed as Exhibit I), lists about 150 presumptively valid patent claims that use the word "including".

Inasmuch as "including" and "comprising" are synonymous, and the noted phrase must be read in context, the present claims should be understood to encompass any recombinant NANBV structural protein or portion that has an enumerated sequence from residue 1 through residue 74 or the entire sequence from residue 1 through residue 120. Thus, there is no indefiniteness to the claims, and this rejection should be withdrawn.

B. Alternative Rejections Under 35 U.S.C. §102(a) and §103

Claims 28, 30, 31, 33 and 36 were rejected as above based on the U.S. patent to Wang. The Action noted that "Wang teaches assaying sera for antibodies against HCV (NANBV) using (a) solid phase coated with synthetic peptides that include amino acid sequences instantly claimed ..." Wang's Example 14 and Table 7 were particularly relied-on, and especially her peptides VIIIE, IXD and IXE. This portion of the Action concluded with a statement to the effect that even if the claimed sequences were recited more narrowly than the "including" language makes them, the method would still be obvious over Wang's teachings because of the similarity of the sequences involved.

This rejection is respectfully traversed for the reasons discussed below.

Before going further, it must be reiterated that the present claims relate to recombinantly produced proteins or portions thereof, whereas Wang teaches the use of synthetically produced polypeptides. Wang teaches at column 24, lines 14-19 that her peptides "were synthesized by the 'classical' Merrifield method of solid phase peptide synthesis using side chain protected t-Boc-amino acids ..."

Although one, a priori, might not think that there would be a difference in an assay result between the use of a chemically synthesized peptide of Wang and a recombinantly prepared similar amino acid residue sequence, the data of the present application, that provided in the accompanying Declaration and that relied-on in the Action show that there is a difference between the chemically prepared and recombinantly prepared materials where the presently claimed assays are concerned. The reason for this difference in result is unknown.

Although the observed difference between a chemically synthesized peptide and a similar recombinantly produced sequence could not be predicted a priori, the Wang patent itself contains a similar situation with two chemically produced peptides. Thus, the sequence EECSQHLPYI is present in both of Wang's peptides I and III. In peptide I, that sequence is shown as being a strong contributor to immunoreaction, whereas in peptide III, the same sequence fails to bind antibodies. See, Wang's Fig. 1-4.

Thus, Wang teaches that the same sequence in two different environments can produce different and unpredictable antibody binding results. The same has been found here in comparing a synthetic peptide to a recombinantly produced protein or portion.

Turning now to the Wang teachings, the Examiner's attention is invited to column 41, lines 45 and 46 of Wang wherein it is stated that the relative percentages of immunoreactivity of Table 7 are related to results obtained with peptide IIID. Wang's Table 1 shows that a relative immunoreactivity of peptide IIID was set at an apparently arbitrary 100 percent, whereas the data of Wang's Fig. 5 show that even with a combination of peptides IIF and IIID, the

resulting synthetic peptide antigen assay did not perform as well as the then industry standard SOD-C100 fusion protein. That latter material is also noted at column 15, line 29, in footnote 18 that cites the Chiron EPO patent application (EPO 0318218AT, 1989). Thus, all of the results of Wang pertinent here are related to results obtained using a peptide that performed as antigen more poorly than did the C100 recombinant non-structural protein.

Turning back to Wang's Table 7 and Example 14, it is seen that the best results obtained were only 98.6 percent as good as that of peptide IIID, and were thus worse than using SOD-C100. Those results were obtained with peptide VIIIE that included residues 2-62 of the capsid protein that correspond to residues 1-62 of Fig. 1 herein. Peptides IXD and IXE (positions 66-120 of the capsid protein), also relied-on in the Action, provided only 49.5 and 58.1 percents, respectively, of the result obtained with peptide IIID, and would thus be still poorer than those with SOD-C100. Peptide IXC that begins at residue 73 and continues through the C-terminal Gly also showed a similar binding value of 57.1 percent, indicating no benefit for antibody binding in the synthetic peptide by the presence of residues 65-73 of the mature protein.

The Examiner's attention is now invited to pages 68 through 73 of the present specification and to Tables 2-6 therein. Comparative data are provided there between various assays that utilized several different assay techniques, of which three are of import here.

The first of those techniques utilized the alanine transferase (ALT) enzyme detection method discussed and cited at page 68, lines 26-29; and used in some of the Wang teachings,

e.g. Example 15. The second utilized the C100 antigen of a commercial kit that corresponds to the SOD-C100 of the Chiron EPO patent application noted in Wang and referred to there as anti-HCV. The third used a recombinant antigen of the present claims designated CAP-N.

The CAP-N antigen contains amino acid residues 1-74 of Fig. 1 herein or residues 1-74 of the structural NANBV protein now referred to as the capsid or core. Construction of that recombinant molecule is discussed at pages 64 through 68. The references there to use of plasmid pGEX-3X-690:694 that relate back to page 50, Table 1 indicate that the plasmid contained DNA that encoded amino acid residues 1 through base 74 (224 bases/3) of the NANBV structural protein.

Turning back to the data between pages 68 and 73 of the present application, it is seen that use of the recombinant CAP-N antigen to bind to NANBV antibodies out-performed the commercial assay kit based on the C100 antigen. For example, Table 2 shows that an assay of the present invention detected antibodies four weeks earlier than did the C100 antigen. Table 3 shows that the C100 antigen never detected antibodies over a 23-week period, whereas a claimed assay detected antibodies at 14 weeks. Table 4 shows that anti-CAP-N antibodies were detected at 4 weeks post infection, whereas the industry standard and the standard against which Wang's peptides were ultimately tested found those antibodies only at 18 weeks. The results of Table 5 are similar, but show a difference only at 2 weeks with the next entry at 40 weeks showing a similar result. The data in Table 6 again show failures by the industry standard where an assay of the present invention showed infection.

Tying the above strands of data together between the Wang disclosures and those of the present inventors, it is seen that Wang in Table 7 and Example 14 at best obtained immunoreactivities poorer than those obtained using the C100 antigen. On the other hand, the present inventors, using their recombinant antigen obtained results that far surpassed those obtained using the C100 antigen.

It is submitted that if a claimed recombinant were the same as, obvious from or equivalent to that of Wang, a similar result should have been obtained between Wang and the present inventors. That similar results were not obtained, and that unexpectedly enhanced results were obtained by the present inventors bespeaks of the unobviousness of a claimed assay that utilizes a recited recombinant. Those unexpected and unobvious results should not go unrewarded and this rejection should be withdrawn.

It should also be noted that Wang's Examples 15-18 illustrate that sensitivities similar to those obtained here with assays based upon a recombinant antigen were not obtained by Wang until mixtures of synthetic peptides from both structural and non-structural (NS) proteins were used. For example, Example 15 of Wang states that "Format C incorporating peptides (IIH, V and VIIIE) from both the HCV structural (core) and non-structural regions was the most sensitive". (Column 43, lines 26-29.) Thus, again the unexpected result obtained with a present core-only recombinant antigen assay as compared to Wang's mixed synthetic peptide assay indicates that a claimed assay has unexpected results, and that this rejection should be withdrawn.

It should also not be argued that the added length of the smallest recombinant herein; i.e., residues 1-74 of Fig. 1, over that of a relied-on Wang peptide is responsible for the

observed difference in immunoreactivity. Enclosed Document BC, further discussed hereinafter, is a paper by the present inventors that shows a recombinant antigen comprising residues 69 through 120 (referred to therein as the CAP-B antigen) failed to react in immunoblot studies with all but one sera from HCV-positive humans and chimps. The one non-negative immunoblot study was itself barely positive, with only a showing of weak binding. Similarly, the Wang patent Table 7 shows that the sequence from residue 65-73 (present in peptide IXD and IXE but not peptide IXC) did not add to immunoreactivity.

Thus, the before-discussed unexpected differences in immunoreactivity, must be due to Wang's use of a chemically synthesized peptide as compared to the present inventors' use of a recombinant protein[portion.]

C. First Rejection Under 35 U.S.C. §103

Claims 32, 34, 35 and 37 were also rejected over Wang. The Action admits that the specific materials whose uses are recited in the dependent claims are not taught by Wang, but are asserted to be well known variations whose use would have been obvious. This rejection is respectfully traversed.

First, as noted above, independent claim 28 and claims 30, 31, 33 and 36 are not obvious over Wang. That being the case, the present claims that all depend from claim 28 should not be obvious either. Thus, this rejection should be withdrawn.

Second, although it is asserted that materials of these claims are well known and that their substitution for the materials of Wang would be obvious, as the Court held in Smithkline Diagnostics, Inc. v. Helma Laboratories Corp., 8 USPQ 2d 1468, 1475 (Fed.Cir. 1988), "one cannot pick and choose among the individual elements of assorted prior art references to

recreate the claimed invention". [Citation omitted.] Here, no references have even been provided for this point and still a picking and choosing has occurred. Thus, again this rejection should be withdrawn.

D. Second Rejection Under 35 U.S.C. §103

Claim 29 was rejected as allegedly obvious over Wang in view of Kuo et al. Kuo et al. (hereinafter Kuo) teaches the production of the recombinant SOD-C100 antigen of the Chiron EPO application and commercial kit used comparatively by the inventors here. The Action asserts that it would have been obvious to prepare a Wang peptide using the Kuo techniques "to gain the advantages of producing peptides by recombinant means, e.g. to obtain a stable, plentiful supply of peptides that are free of contamination of other HCV peptides." This rejection is respectfully traversed.

It is first submitted that inasmuch as independent claim 28 has been shown to be non-obvious, as discussed above, claim 29 that depends from claim 28 can also not be obvious. Thus, this rejection should be withdrawn.

Second, it is submitted that the best way to make a peptide free of other HCV antigens, host cell antigens as well as other possible antigens is to do what Wang did, build it by chemical syntheses. Such chemical syntheses using only organic solvents and t-Boc-blocked amino acids assure an absence of related antigens. Thus, the conclusion reached for using a Kuo technique is incorrect and this rejection should be withdrawn.

Third, Kuo used yeast to make his recombinant. Yeast typically exclude inserted plasmids after several generations and

are not a "stable" source of a recombinant. Again, this rejection should be withdrawn.

Fourth, it is far more difficult to obtain a useful recombinant than it is to obtain a chemically produced, synthetic peptide as did Wang. The work-up of cell lysates required to obtain the desired recombinant protein or fusion protein is typically far more arduous than is the work-up from peptide synthesis, where programmed machines do most of the work and an HPLC separation of the cleaved, deblocked peptide can provide the useful material. It is further understood that expression in yeast cells as done by Kuo is usually not as efficient as expression in E. coli, thereby making purification still more difficult. The rejection should be withdrawn.

III. THE PRINCE DECLARATION

Also enclosed herewith is a Declaration of Dr. Alfred M. Prince. Dr. Prince is the leader of the New York Blood Center research group involved with this application and a named inventor herein. As noted by the Wang research group in the first line of enclosed Document BA that is discussed hereinafter, it was Dr. Prince who named NANBV as hepatitis C virus.

Dr. Prince's Declaration provides data that illustrate efficacy of a claimed assay based on the whole recombinant capsid protein from amino acid residue position 1 through 120 of Fig. 1. Table 1 of Dr. Prince's Declaration provides exemplary data similar to the data of pages 68-73 of the present specification for assays using the above recombinant protein that contains the capsid 1-120 sequence. Those data show optical density values for the C100 and recombinant antibody binding studies for nine transfusion patients whose sera tested negative in a C100-based assay and which sera were found positive using the recombinant 1-120 region antigen. Those data, like the data for the CAP-N

recombinant illustrate that that claimed recombinant is also more immunologically sensitive than the C100-based assay and detected antibodies after a period of months in which the latter assay continued to show negative results as to infection.

Thus, the unexpected result obtained using the CAP-N recombinant is also observed using the 1-120 recombinant. Both were more sensitive than the C100-based assay, which itself was more sensitive than that shown using peptide VIIIE as antigen as is disclosed in Wang's Table 7 and Example 14.

Dr. Prince's Declaration continues with a discussion of his studies of assay kits provided by Dr. Wang's associates at United Biochemical, Inc. (UBI), the assignee of the Wang patent. Three types of kits were provided that were labeled "ST", "NS" and "HCV". Although the specific antigens in each were not identified, Dr. Prince was informed by Dr. Barbra Hosein of UBI that the kit labeled "ST" contained synthetic peptide from a structural protein, that labeled "NS" contained non-structural protein synthetic peptide, and that labeled "HCV" contained synthetic peptides from structural and non-structural proteins. He presumed that those three kits contained the antigens of the assays described in the Hosein et al. article that is enclosed as Document BA.

Inasmuch as Dr. Prince's Declaration and the discussion in IV, below, correlate the data between the Hosein et al. paper and data of the Wang patent for Formats A and C, and the UBI kits Dr. Prince's group used are presumably those of the Hosein-et al. paper, above, identification of the antigens in the UBI kits provided to Dr. Prince is possible. The kit labeled "ST" contained peptide VIIIE, that labeled "NS" contained peptides IIH and V, and that labeled "HCV" contained all three.

The kit labeled "ST" and containing peptide VIIIE was used for the comparative studies of Table 2 of Dr. Prince's Declaration, whereas the kit labeled "HCV" containing all three peptides was used for studies in the enclosed Sugitani et al. paper that is referred to herein as Document BB and is discussed hereinafter.

The data in Table 2 of Dr. Prince's Declaration show pertinent data for a chimpanzee designated Chimpanzee No. 10 inoculated with virus in 1977 and from which blood samples were taken and stored over a period of several years. The data of Table 2 show that for that chimp, the claimed assay based upon the recombinant capsid 1-120 sequence was able to detect infection whereas the UBI-ST kit based on the Wang peptide VIIIE as antigen showed no evidence of infection during the acute phase of the infection nor during the chronic phase.

Thus, again, the unexpected advantage of using an assay based on a recombinant antigen of the invention over a similar chemically produced antigen was shown.

IV. FURTHER ART

Five papers of possible interest here, at least four of which were published after the filings of both Wang and the present application, have come to counsel's attention and are noted here to complete the record and underscore that which has already been discussed. The first paper published is by Wang and her co-workers [Hosein et al., Proc. Natl. Acad. Sci. USA, 88:3647-3651 (May 1991)]. The second is by two of the present inventors and their co-workers [Sugitani et al., Lancet, 339:1018-1019 (April 1992)]. The third, by inventors herein [Nasoff et al., Proc. Natl. Acad. Sci. USA, 88:5462-66 (1991)] was published prior to the Wang paper. The fourth paper is

Okamoto et al., Japan. J. Exp. Med., 60:222-233 (1990), whereas the fifth is Okamoto et al., Hepatology, 15:180-186 (1992).

Copies of the above papers are enclosed herewith as documents BA, BB, BC, BD and BE, respectively. They are also noted on enclosed Form PTO-1149.

The first paper (BA) discusses assays run using chemically synthesized peptides. An unidentified capsid (core) peptide "selected from a region covered by amino acids 1-120" was used as the single antigen in EIA I, peptides from two non-structural proteins were used in EIA II and all three peptides were used in EIA III. These three formats are thus similar to Formats A, B and C/D of the Wang patent.

Although there is not an exact identity of data (presumed to be due to the typographical errors because of the complete identity of the remaining data), it is believed that the data of Table 1 of this paper for donor 1 are the same as those of Table 8 of the Wang patent for panel 1. Similarly, the results of the second paragraph on the left side of page 3649 for Japanese dialysis patients can be obtained by ready calculation from the data of the Wang patent Table 9. That being the case, the peptides of EIA II correspond to those of Format A of the Wang patent, whereas the EIA III peptides are those of Format C of the patent that used peptides IIH, V and VIIIE. Inasmuch as EIA III is said in the paper to contain all three peptides of EIA I and EIA II, the peptide of EIA I must have been peptide VIIIE of the patent.

This paper discusses the added sensitivity of anti-HCV antibody detection when a capsid synthetic peptide is added to peptides from non-structural proteins, including earlier detection of seroconversion as compared to the C-100 antigen-

based assays. Missing, however, are data for the capsid synthetic peptide alone; i.e., peptide VIIIE.

The second paper (BB) compares various assays that include a Wang group kit (UBI-HCV, reference 5) an assay of the present invention (Capsid) and C100 kit used for comparison herein (C100-3). The data of the table show that an assay of the present invention based on a recombinant capsid corresponding to residues 1-120 was equally sensitive to the UBI-HCV kit containing three peptides and a second generation kit from Abbott (Abbott-II) that contains two non-structural antigens and a capsid antigen. All three identified 13/19 or 68 percent of the PCR-positive sera.

Thus, another unexpected result is found here. An assay of the claims based on a single recombinant whole protein (Fig. 1., residues 1-120) was as sensitive as an assay based on a mixture of three chemically synthesized peptides from three different proteins.

Enclosed paper three (BC) describes the CAP-N antigen used in the present application. Although the nomenclature is different, it is apparent that the capsid antigen designated CAP-A of BB is the CAP-N antigen of the present application.

Document BD is an apparent follow-up to the Okamoto et al. paper of record herein that is cited twice in the paragraph bridging pages 1 and 2 of the present application. This paper deals with the use of a 36-mer synthetic peptide that contains residues 39-74 of the HCV capsid as an antigen in an assay for anti-HCV antibodies.

The first page of the article indicates that it was received for publication on June 13, 1993. A computer-assisted search in the MEDLINE data base of DIALOG Information Services, Inc., indicates that Document BD was published in August of 1990.

The mailing and receipt dates of this article are unknown, but are being sought from counsel's Japanese associates and will be provided to the Examiner on receipt.

As is seen from the Summary, the anti-synthetic peptide assay (anti-CP9) and the commercial anti-HCV assay overlapped with positive results in 54 percent of 324 cases of acute or chronic NANB liver disease, with 18 percent of the sera being positive only in the anti-CP9 assay and another 15 percent of the sera being positive in the anti-HCV assay and negative in the anti-CP9 assay, leaving another 13 percent undetected in either assay.

Document BE published in 1992 is an apparent follow-up to Document BD. Here, another synthetic peptide was used in the assays. That peptide was designated CP10, and includes 19 residues covering amino acid residue positions 5-23 of Fig. 1 herein. It is noted that this paper used the two peptides separately and summed the results obtained from separate assays rather than linking the peptides or using a mixture of both in the assays.

V. SUMMARY

The specification has been amended to correct obvious errors in typing and the claims have been amended to recite use of only recombinant antigens. Each of the bases for rejection has been dealt with and overcome or otherwise made moot. A Declaration of one of the inventors is enclosed that provides further data for a claimed recombinant as well as information regarding data by the inventors and Dr. Wang and her research group that were published subsequent to the filing dates of this application and the Wang patent. Copies of those papers are enclosed.

It is therefore believed that the application is in condition for allowance. An early notice to that effect is earnestly solicited.

Respectfully submitted,

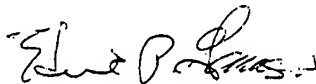
By 
Edward P. Gamson, Reg. No. 29,381

Enclosures

Petition and fee
Prince Declaration and enclosure
Further art (BA-BE)
Form PTO-1449
Exhibit I

CERTIFICATE OF MAILING

I hereby certify that this Amendment Under 37 C.F.R. §1.115, together with the stated enclosures, is being deposited with the United States Postal Service as First Class Mail, postage prepaid, in an envelope addressed to: Hon. Commissioner of Patents and Trademarks, Washington, D.C. 20231 on January 28, 1993.



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Matthew Bender

(Matthew Bender & Co., Inc.)

**Times Mirror
Books**

(Pub. 346)

ENDNOTES in Part III.]

angle of incidence⁸⁸⁴

beam incident on said first end surface⁸⁸⁴
 illuminating device¹⁷¹⁸

scattered intensity, scatters the incident light¹⁷¹⁸

vents⁸⁰⁰¹

ation¹⁸⁶⁸

the incisal end to the gingival end¹⁷⁸⁸

axial and transverse inclinations of the

g incline with curve maxima (32,734) and curve

ections inclined²⁶²⁴

1467

I-10

(Ref. 7-3/92 Pub. 546)

Include

[Superscript References are to ENDNOTES in Part III.]

Include

as well as competing ions including calcium and/or magnesium⁸⁰⁵⁴

housing including means for flowing blood constituents . . . the rotor including
 inner and outer walls . . . the rotor including input ports . . . said means
 including platelet concentrate ports . . . said means including apertures⁸⁰⁵⁰

elevator means including a lift tower . . . said lift car including a pair of laterally
 spaced movable fingers⁷⁶⁴¹

including at least one . . . track element . . . including an integrally molded
 fastening tongue element . . . including means defining a receiving slot . . .
 including an elongated . . . main portion⁷⁸¹⁷

including those having alkyl, aryl, or cycloalkyl substituents⁸⁰⁴⁴

a catalyzing agent including manganates and permanganates⁸⁰²³

rectangular housing including mutually opposed side walls . . . said roof including
 means for attaching said base panel . . . said evaporator unit including damper
 means . . . said damper means including inside temperature sensing means⁸²⁷⁸

movement along a plurality of axes including a vertical axis⁸³⁰⁶

smelling substances, including onions, garlic, sugar substitutes including sorbit,
 xylitol, and mixtures thereof⁷⁷⁸²

which includes⁸²²⁶

said method including passing an electric current⁸³⁶⁴

ambient conditions which include¹⁹⁰³

include an end blade¹⁷⁴⁸

said housing includes a body member . . . including a valve member¹⁸⁰⁹

including as a cross-linking agent in said composition⁸⁷¹

a citrus half including the rind⁸⁷²

R may represent lower alkylene including methylene⁸⁸⁸

including compounds wherein NR¹R² . . . is . . . piperidino, pyrrolidino⁸⁹

a water level indicator . . . including movable means²⁰

said composition including a blowing agent¹⁰⁶

a process . . . including the steps of⁷⁷³

means including a photodetector for sensing⁸⁷⁴

apparatus . . . including . . . filled with a liquid including . . . said area varying
 means includes . . . said frangible means for shielding including⁸⁷⁹

the method which includes⁸⁷⁸

sheets including a pattern of gray monotone effects⁸⁰¹

including in the gas a coating substance⁸⁷⁷

INCLUDE: Although this term is used in claims of a number of issued patents, it may be regarded
 as introducing an element of indefiniteness. A better view appears to be that the "including" phrase
 adds further, although not exclusive, definition.

Cf. Rosemount, Inc. v. Beckman Instruments, Inc., 221 U.S.P.Q. 1 (Fed. Cir. 1984); *Studiengesell-*
schaft Kohle, m.b.H. v. Dart Industries, Inc., 220 U.S.P.Q. 841 (Fed. Cir. 1984); *Gould v. Mossing-*
hoff, 215 U.S.P.Q. 310, 313 (D.C. D.C. 1982); *Ex parte Schaefer*, 171 U.S.P.Q. 110 (P.T.O. Bd. App.
 1970).

By the use of the word "including" the combination may have elements other than those recited.
Ex parte Russell, 153 U.S.P.Q. 752 (P.T.O. Bd. App. 1966).

I-10.1

Quincy Reader & Co., Inc.)

(Ref. 7-3/92 Pub. 546)

Include

[Superscript References are to ENDNOTES in Part III.]

Include—Cont.

the solution portions include neither the uppermost nor the lowermost quantity of the solution mass⁸⁰⁴
fuel assemblies including elongated openings extending . . . mechanisms including operating means for . . . moving said . . . drive means⁸⁷⁹
annular mould cavities including the mould cavity for the tread portion⁸⁴⁵
a plane including the longitudinal axis⁸⁷⁹
said surfaces including a surface between said flanges⁸⁴⁴
said means including a fixed diameter portion⁸⁴⁷
techniques including exposing the first layer⁸⁰²⁰
including fiber material in impregnation liquid⁷⁹⁰
during normal reactor operation including load follow⁸⁸⁰
said second flow loop including a fluid pump⁸⁸¹
including the steps of⁸⁰⁴
said anode including a substrate coated with⁸²³
product including normally liquid hydrocarbons⁸⁰⁹
product including ethylene . . . includes solids derived from⁸⁴⁷
vibrating assembly including a screen⁸⁴⁹
certain gaseous hydrogen compounds, including ammonia⁸⁸³
including a regenerator⁸⁸⁴
cement mix including hydraulic cement, aggregate, sufficient water⁸²⁸
electrode including a tubularly shaped body⁸⁹⁹
a catalyst which includes alumina⁸²⁰
monomers including at least 3% by weight of⁸⁹¹
and including a continuous interior wall⁸⁰⁹
a machine control including a memory⁸¹⁰⁹

(Text continued on page 4)

Include

(Superscript References are to ENDNOTES in Part III.)

lowermost quantity
mechanisms including
read portion
n 667
at water
continued on page I-1

including a stop²⁶²³
including a filter means treatment substance²²⁸⁴
including a guide includes a pulley¹⁸⁴³
including a worm drive²⁴⁴³
including a differential amplifier means²⁸⁰³
including a generator includes⁶⁰⁶⁸
including at least partial traversals²⁵⁴³
including a lesser portion⁴⁹⁵³
including hold-open means³²⁴⁴
including a tread face²⁷⁷³
including a main frame²²⁴⁴
including an angle¹⁸²⁴
including a pair of connectors²²⁹¹
including a band adapted to releasably encircle¹⁸²⁶
including a side member¹⁴³⁷
including means for defining entrance and exit passages¹⁴⁴⁰
including a chute-like area¹⁴⁴³
including a housing . . . including an insert¹⁸²⁹
including a housing¹⁸²⁹
including two branches²³²²
including outlets¹⁸³³
including agronomically acceptable salts, esters and amides¹⁸³⁴
including means adapted to permit a milk flow¹⁴⁸⁴
including a pair of pivoted jaw members¹⁴⁹⁴
including in said mixture¹⁸⁴⁴
including a plenum chamber¹⁸⁶⁸
including, in combination¹⁸⁸³
including a plurality of coolant channels¹⁸⁸⁵
including a surface portion¹⁸⁸⁶
including a pusher member . . . including input presser means . . . including
discharge presser means¹⁸⁷⁴
including stretching means . . . including means for varying the amount of
elongation¹⁸⁸⁰
including a DC voltage regulator . . . including AND gates . . . including a
plurality of subroutines¹⁸⁸⁶
pump including¹⁸⁹⁰
including a relief port means¹⁸⁹¹
including¹⁸⁴⁸
including a housing structure adapted to be installed . . . including a bearing¹⁸⁹⁷
including means¹⁸⁹⁰
including register means¹⁸⁹²
including an opening aligned with²⁸⁰³
including structure defining²⁸⁰⁷
including a collet portion²⁸⁰⁸

Include

[Superscript References are to ENDNOTES in Part III.]

Include—Cont.

including a deflector for deflecting said engaging means²³¹⁸
said apparatus including a support¹⁶¹⁰
including a first means for conveying articles . . . including a second
means²⁷⁹²
including a plurality of bearings . . . including a plurality of rollers¹²²
the device including . . . the top region including¹⁶¹³
including a rounded annular juncture²²²⁷
including drawing from readily bendable sheet metal²²²⁷
including a longitudinally extending bore²³⁰⁴
including a resistance²²⁷⁰
including a plurality of parallel slats fixedly secured . . . including inter-
means²³²⁹
including a counter²⁴⁴²
including a plurality of holding cribs . . . including a sensor-actuated
device¹⁶⁴²
a reel including a spool²³³⁹
including one-side facing¹⁶⁴⁶
including a frame¹⁶⁴⁸
a clip including a hook portion engageable with said rail²²⁴⁰
including a magnetic member²²⁷⁸
including a movable valve closure element²²⁷⁸
including self aligning means²²⁷⁸
including a lower surface . . . including a preload spacer²²⁷⁸
metals of groups IV-B, V-B, and VI-B of the periodic system, including
and uranium²²⁶⁶
including at least two rotating electrical contact sheaves²²⁰²
including in combination therewith²²⁰²
including stationary sprocket²²⁰²
including a punch²²⁰⁷
including engaging means²²⁰⁹
including a single bore¹⁶⁴²
including two cords²²⁰⁹
each jaw mechanism including²⁶¹⁶
including laterally spaced support frame members^{2626, 2626}
including windings, a magnetic circuit and a supporting casing²¹¹²
including a hanger²⁶²⁸
including a polymeric material¹⁶⁹⁹
including rotatable platform means¹⁶⁶¹
including biasing means²⁶³⁴
including a common electrode¹⁷²⁰
an object sub-unit including a visual object¹⁷²⁴
including a rotatable member . . . including a clamp member²⁰⁹⁶
a housing including a skirt¹⁷²⁴
including a simulated image²¹⁹⁷

ES in Part III.]

[Superscript References are to ENDNOTES in Part III.]

Cont.

ans⁷⁵¹⁸

including a second lock

rality of rollers⁸⁸³

13

1⁸⁸²⁷

... including integ

; a sensor-actuated op

d rail⁸⁴⁸spacer²⁵⁷⁵

lic system, including the

eaves²⁸⁸³

626, 2626

rting casing⁸¹¹²np member⁸⁰⁹⁶including means for receiving¹⁷³⁹including upper and lower main journals¹⁷⁴²including energy distribution means¹⁷⁴⁶including longerleg, shorterleg therebelow⁸⁸⁸⁸including, a resilient planar portion¹⁷⁷¹

including: a plunger valve assembly . . . including: said means for forming . . .

including said shaft portion . . . including a front end¹⁷⁷⁸including the steps of¹⁸⁴⁷administering to a mammal, including a human³⁷⁷⁶including a plurality of pots¹⁷⁷⁹including at least one central channel¹⁷⁸⁰

including cross braces . . . including a shaft . . . including vertical upright

telescoping handle portions¹⁷⁸²including a body¹⁷⁸³including tin chloride . . . plating electrolessly a layer including nickel⁴⁹⁷⁵including a dam extending transversely of⁴⁹⁶⁸including at least one silver halide emulsion layer³²⁸³including injectors⁴⁹⁶⁶including at least a first preheater stage¹⁷⁸⁷including a support frame¹⁷⁸⁸including clocking means¹⁷⁸⁴including structure¹⁷⁸⁷including a hub¹⁸⁰¹including crosslinker, antioxidant and pigments³³⁰³including within the polymerizable stream a minor proportion³³⁰⁴

a thermoplastic basic plastic, including polyethylene, polypropylene or polyvinyl

chloride³³⁷⁰liquid products including gasoline³⁹²²including a belt means¹⁸¹²

including an access-port-defining structure . . . including a lower end . . .

including a through bore¹⁸¹⁴

Inclusion

a three-dimensional molecular structure with inclusion ability⁷³⁹⁹an insoluble, impure inclusion body in a transformant microorganism⁶⁸⁷³inclusions and inclusion clusters . . . entrapping inclusions¹⁸²⁸inclusion of slag²⁵⁸⁴

Inclusive

m is an integer from 1 to 4 inclusive⁷²⁴⁸

Incoming

an incoming digital data signal¹⁶³⁴

Incompatible

incompatible with³²⁰⁸

#17
LP
2-12-93

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee et al.)
Serial No.: 07/573,643) Attorney Docket
) PHA 0025P
Filed: August 27, 1990) Group Art Unit: 1802
For: NON-A, NON-B, HEPATITIS VIRUS)
ANTIGEN, DIAGNOSTIC METHODS AND)
VACCINES)
Examiner: D. Wortman)

DECLARATION OF ALFRED M. PRINCE, M.D.

RECEIVED
FEB 1 1993

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

ALFRED M. PRINCE, M.D., Declares

1. That he is the Alfred M. Prince who is a named co-inventor of the subject matter of the above-identified patent application;

2. That he is employed by the Lindsley F. Kimball Research Institute, a Division of The New York Blood Center (NYBC), an assignee of the above-identified patent application;

3. That a true and accurate copy of his Curriculum Vitae entitled "Biographical Sketch" is attached hereto that lists, inter alia, his educational background, work history, awards and the almost three hundred published papers and book chapters of which he is a sole or joint author, including approximately thirty-seven publications relating to hepatitis C virus;

4. That he has read and is familiar with the outstanding Office Action on the above-identified patent application, the art relied-on in that Action, and the Response filed herewith;

5. That he is the head of the Laboratory of Virology at the NYBC and is the leader of the NYBC research group that carried out the work at that institution related to this patent application;

6. That he and those under his direction and control have continued work on the invention defined by the presently amended claims;

7. That as part of that continued work, a number of serum samples were obtained from transfused human patients that were screened for anti-HCV antibodies using a commercially available assay that contained the recombinant C100 antigen and were found to not contain such antibodies;

8. That those sera were also screened in an assay of the amended claims using a recombinant antigen containing residues 1-120 of application Fig. 1 as the only antigen, and about 5 to about 10 percent of those sera were found to contain antibodies that bound to the recombinant antigen;

9. That not only were those previously negative anti-HCV antibody-containing sera found to be positive for the presence of those antibodies in a presently claimed assay, but in three exemplary instances, an assay of the present invention was positive for the presence of those antibodies over an infection period of one to three months during which time the C100-based assay showed the sera to be negative;

10. That the optical density values obtained for the sera from nine C100 assay-negative patients discussed in Paragraphs 8 and 9, above, are shown below in Table 1, in which the numbers at the left show the patient number, the "week" is the week post transfusion, "C-100" is the observed optical

density using that assay with the "-" sign thereafter indicating a negative assay for anti-HCV antibodies, and "CAP" being the optical density values obtained using the recombinant 1-120 residue capsid antigen with the "+" sign indicating a positive response in the assay;

Table 1

Transfusion Patient
Sera Positive for Antibody to CAPSID
1-120 and Negative for Antibody to C-100

<u>Patient #</u>	<u>WEEK</u>	<u>C-100</u>	<u>CAP</u>
9	4	0.08-	0.47+
18	7	0.18-	1.93+
18	10	0.2-	1.88+
18	17	0.12-	1.91+
92	21	0.12-	1.33+
92	22	0.03-	1.59+
92	24	0.18-	1.33+
117	10	0.09-	0.48+
169	14	0.12-	1.1+
169	16	0.36-	1.1+
169	19	0.14-	1.88+
169	21	0.11-	1.69+
169	23	0.17-	1.74+
201	8	0.12-	0.6+
213	13	0.24-	0.81+
257	10	0.25-	0.88+
299	8	0.09-	0.46+

11. That in a further aspect of his research group's work with the present invention, he was provided with three solid phase assay kits by Dr. Barbra Hosein, one of Dr. Wang's associates at United Biochemical, Inc. (UBI), the kits being labeled "ST", "NS" and "HCV";

12. That he was not informed of the specific antigen utilized in each kit, but he was informed that the kit labeled "ST" contained synthetic peptide from a structural protein, that labeled "NS" contained synthetic peptide from non-structural protein, and that labeled "HCV" contained synthetic peptide from both structural and non-structural proteins;

13. That he presumed that the peptide antigens of those kits were those described in Hosein et al., Proc. Natl. Acad. Sci. USA, 88:3647-3651 (1991) that is enclosed with the accompanying Response as Document BA;

14. That the Hosein et al. article (Document BA) does not specify the synthetic peptide antigens used by sequence position, but the substantial identity of the data in Table 1 of Document BA with those of the Wang patent Table 8, panel 1, the identity between the results discussed on the left side of page 3649 of Document BA and the data of Table 9 of the Wang patent, and his presumption of Paragraph 13 permit him to come to a belief as to the identities of the specific synthetic peptide antigens used in Document BA and in each kit he received;

15. That it is his belief that the synthetic peptide antigens used in the kits he received, the Hosein et al. paper (Document BA) and the Wang patent are as shown below:

<u>UBI Kit</u>	<u>Hosein et al. (Doc. BA)</u>	<u>Wang Patent Format</u>	<u>Wang Patent Peptide</u>
NS	EIA II	A	IIH & V
HCV	EIA III	C	IIH, V & VIIIE
ST	EIA I	---	VIIIE

16. That enclosed with the Response as Documents BB and BC are true copies of two papers published after the filing date of the above-identified application that are authored by him and his co-inventors and co-workers;

17. That with one exception, the data and disclosures of those two papers were believed at the times of their submission, publication and are now believed to be true and correct;

18. That the one exception in Paragraph 17, is that footnote 6 (to Document BC) was cited in error in that the results reported for the "Capsid" of the table of Document BB

were obtained using a recombinant protein containing amino acid residues 1-120 of application Fig. 1 as in Table 1, above in Paragraph 10, rather than a shorter recombinant of Document BC;

19. That the results shown in the table of Document BB illustrate that use of the single recombinant protein containing residues 1-120 of Fig. 1 of this patent application were the same as those obtained using the UBI-HCV (presumed Wang synthetic peptides IIH, V and VIIIE) assay and were better than those obtained using the C100 antigen-based assay;

20. That in still further work related to the present invention, results obtained using an assay based on the claimed recombinant 1-120 residue sequence antigen were compared with results obtained using the UBI kit labeled "ST" (the kit believed to use Wang patent VIIIE as antigen), using sera obtained from chimpanzees infected with HCV and from which blood samples were taken and stored over a period of years;

21. That the results for those sera were comparable with the exception of the sera from a chimpanzee designated Chimpanzee No. 10;

22. That chimpanzee No. 10 was inoculated with HCV in November of 1977, with blood samples being taken throughout 1978 and thereafter; the animal being rechallenged with HCV during the sample-taking time period;

23. That pertinent data related to the serum samples from Chimpanzee No. 10 are provided below in Table 2, whose entries have the following meanings: Date = date data were taken; Week = week prior or subsequent to inoculation with HCV; HIST = histological evaluation of liver tissue biopsy, in which NORM means a normal appearance, NSRH means non-specific reactive hepatitis, AH means acute hepatitis, and CPH means chronic

persistent hepatitis; CAP the optical density (O.D.) reading using an assay based on the recombinant 1-120 sequence of Fig. 1 as antigen, with an O.D. of 0.35 or greater indicating a positive result, and N indicating a negative result; and UBI-ST = O.D. values obtained using a provided UBI kit designated "ST", with the dash after the number indicating a negative finding.

Table 2Data for Chimpanzee No. 10

<u>Date</u>	<u>Week</u>	<u>HIS</u>	<u>CAP</u>	<u>UBI-ST</u>
11-08-77	-2	NORM	N	0.01-
12-27-77	6	NORM	N	0.05-
02-07-78	12	NSRH	N	0.04-
03-21-78	18	AH	0.52	0.01-
05-02-78	24	AH	0.49	0.01-
06-13-78	30	AH	0.48	0.00-
07-25-78	36	CPH	N	0.01-
11-09-78	51	CPH	---	0.02-
11-23-78	53	CPH	0.47	---
12-04-78	55	CPH	---	---

24. That the data of the studies shown and particularly Table 2 show that a claimed assay utilizing a recited recombinant as the sole antigen performed better than did an assay based on a single synthetic peptide having most of the same sequence, in that an assay of the present claims detected HCV infection in both the acute and chronic forms, whereas the assay based on the similar synthetic peptide detected neither type of infection;

25. That he further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may

Serial No. 07/573,643

-7-

jeopardize the validity of any patent issuing on this application.

Date 1-27-93 Alfred M. Prince
Alfred M. Prince, M.D.

Enclosure

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington D.C. 20231,

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EDWARD P. GAWSON
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Edward P. Gawson
SIGNATURE

January 28, 1993
DATE OF SIGNATURE

BIOGRAPHICAL SKETCH

Name: Alfred M. Prince, M.D.
 Title: Senior Investigator
 Birthdate: December 16, 1928

INSTITUTION AND LOCATIONS	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Yale University New Haven, Connecticut	B.A.	1949	Premedical
Columbia University, New York, New York	M.A.	1951	Microbial Genetics
Western Reserve University School of Medicine Cleveland, Ohio	M.D.	1955	Medicine- Virology
University of Tampere, Finland	M.D. (Hon.)	1981	Medicine

- 1950 - 1955 Western Reserve University School of Medicine: studies on Newcastle Disease Virus, with Dr. Harold S. Ginsburg.
- 1955 - Borden Award for Undergraduate Research, AOA.
- 1955 - 1957 Internship and assistant residence in pathology, Yale University School of Medicine. Sigma Xi, 1956. Fellow of the American Cancer Society.
- 1955 - 1959 Yale University: independent investigations on Rous Sarcoma Virus.
- 1957 - 1959 Instructor in pathology, Department of Pathology, Yale University School of Medicine.
- 1959 - 1962 Chief, Department of Virus and Rickettsial Diseases, Medical General Lab, U.S. Army, Tokyo studies on Viral Hepatitis, Japanese Encephalitis and Epidemic Hemorrhagic Fever.
- 1962 - 1963 Associate Member, Wistar Institute: studies on Rous Sarcoma, Epidemic Hemorrhagic Fever, and Hepatitis Viruses.
- 1963 - 1964 Assistant Professor, Department of Pathology, Yale University School of Medicine.
- 1964 - Present Diplomate of American Board of Pathology in Anatomic Pathology.
- 1966 - Present Clinical Associate Professor of Pathology, Cornell Medical College.
- 1965 - 1972 Investigator and Head, Laboratory of Virology, The New York Blood Center.
- 1967 - 1975 Career Scientist, Health Research Council of the City of New York.
- 1975 - The Karl Landsteiner Award of the American Association of Blood Banks.
- 1972 - Present Senior Investigator, and Head Laboratory of Virology, The New York Blood Center.
- 1976 - Present Chairman, International Task Force for HBV Immunization.
- 1988 - Alpha Award.

1. Prince, A.M., Ginsberg, H.S.: Immunohistochemical studies on the interaction between Ehrlich ascites tumor cells and Newcastle disease virus. *J. Exptl. Med.* 105:177-188, 1957.
2. Adams, W.R., Prince, A.M.: An electron microscope study of the morphology and distribution of the intracytoplasmic virus-like particles of Ehrlich ascites tumor cells. *J. Biophys. Biochem. Cytol.* 3:161-170, 1957.
3. Prince, A.M., Little, A.S., Ginsberg, H.S.: Methods for quantitative bioassay of Ehrlich ascites-tumor cells. *J. Nat. Cancer Inst.* 18:487-506, 1957.
4. Prince, A.M., Ginsberg, H.S.: Studies on the cytotoxic effect of Newcastle disease virus (NDV) on Ehrlich ascites tumor cells. I. Characteristic of the virus-cell interaction. *J. Immunol.* 79:94-106, 1957.
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6. Adams, W.R., Prince, A.M.: An electron microscopic study of incomplete virus formation. Infection of Ehrlich ascites tumor cells with chick embryo-adapted Newcastle disease virus (NDV). *J. Exptl. Med.* 106:617-626, 1957.
7. Prince, A.M.: Quantitative studies on Rous sarcoma virus. I. The titration of Rous sarcoma virus on the chorio-allantoic membrane of the chick embryo. *J. Nat. Cancer Inst.* 20:147-159, 1958.
8. Prince, A.M.: Quantitative studies on Rous sarcoma virus. II. Mechanism of resistance of chick embryos to chorio-allantoic inoculation of Rous sarcoma virus. *J. Nat. Cancer Inst.* 20:843-850, 1958.
9. Prince, A.M.: Quantitative studies on Rous sarcoma virus. III. Virus multiplication and cellular response following infection of the chorio-allantoic membrane of the chick embryo. *Virology* 5:435-457, 1958.
10. Prince, A.M.: Quantitative studies on Rous sarcoma virus. IV. An investigation of the nature of "non-infective" tumors induced by low doses of virus. *J. Nat. Cancer Inst.* 23:1361-1381, 1959.
11. Prince, A.M.: Studies on the mechanism of induction of neoplasia by Rous sarcoma virus. *Acta Union Internationale Contre le Cancer* 15:832-836, 1959.
12. Prince, A.M.: Quantitative studies on Rous sarcoma virus. V. An analysis of the mechanism of virulence of the Bryan "high titer" strain of RSV. *Virology* 11:371-399, 1960.
13. Prince, A.M.: Quantitative studies on Rous sarcoma virus. VI. Clonal analysis of in vitro infections. *Virology* 11:400-424, 1960.
14. Sakurada, N., Prince, A.M.: Characteristics of the disc assay for enterovirus antibody. *Jap. J. Exptl. Med.* 31:179-186, 1961.

15. Prince, A.M.: Factors influencing the determination of cellular morphology in cells infected with Rous sarcoma virus. *Virology* 18:524-534, 1962.
16. Prince, A.M.: Antigenic variation in Asian influenza virus. *Medicine (Japan)* 19:231-234, 1962.
17. Prince, A.M.: Tumor virus: cell relationships. A critical survey of findings in the polyoma system. *Progr. Med. Virol.* 4:208-258, 1962.
18. Hashimoto, N., Prince, A.M.: Kinetic studies on the neutralization reaction between Japanese encephalitis virus and antiserum. *Virology* 19:261-272, 1963.
19. Tomita, Y., Prince, A.M.: Photodynamic inactivation of arbor viruses by neutral red and visible light. *Proc. Soc. Exptl. Biol. Med.* 112:887-890, 1963.
20. Prince, A.M., Taguchi, F.: Studies on serological homogeneity of Asian influenza strains. *Proc. Soc. Exptl. Biol. Med.* 113:766-770, 1963.
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Comparable to
Form PTO-1449
(Rev. 5/92)

U.S. Department of Commerce
Patent and Trademark Office

Atty. Docket No.
PHA-0025P

Serial No.
07/573,643

INFORMATION DISCLOSURE CITATION
(Use several sheets if necessary)

Applicant
Zebdoo et al.

Filing Date
August 27, 1990

Group
1802

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

BA .. Hosein et al., Proc. Natl. Acad. Sci., USA, 88:3647-3651 (May 1991) ..

BB .. Sugitani et al., Lancet, 339:1018-1019 (April 1992) ..

BC .. Nasoff et al., Proc. Natl. Acad. Sci., USA, 88:5462-66 (1991) ..

BD .. Okamoto et al., Japan. J. Exp. Med., 60:222-233 (1990) ..

BE .. Okamoto et al., Hepatology, 15:180-186 (1992) ..

Examiner

Date Considered

*Examiner: Initial if citation considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

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Washington, D. C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
573643	8-25-90	Zebedee	PHA-0025P

EXAMINER	
Wortman	
ART UNIT	PAPER NUMBER
1802	18

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

- (1) Examiner Wortman (3) T. Helting
(2) E. Gamson (4) _____

Date of interview 2-23-93

Type: ☐ Telephonic ☒ Personal (copy is given to ☐ applicant ☒ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☐ No. If yes, brief description: _____

Agreement ☐ Was reached with respect to some or all of the claims in question. ☒ Was not reached.

Claims discussed: _____

Identification of prior art discussed: Wang US Patent 5106726

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Wang Patent

was discussed and declaration of Dr. Prince was
also discussed. Mr. Gamson will attempt to obtain
more information about the comparison of prior art
and impact method. Supplemental arguments/precedence
amendment will be submitted.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1-7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☒ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Charles Wortman
Examiner's Signature

#20
2-26-93

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee et al.)
Serial No.: 07/573,643) Attorney Docket
) PHA 0025P
Filed: August 27, 1990) Group Art Unit: 1802
)
For: NON-A, NON-B, HEPATITIS VIRUS)
) ANTIGEN, DIAGNOSTIC METHODS AND
) VACCINES
)
Examiner: D. Wortman)

Supplemental Response

RECEIVED

FEB 16 1993

GROUP 1800

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

This is to supplement the Response filed on January 28, 1994 for the subject application.

A. Document BD

Document BD supplied with the prior Response bore a publication date of August 1990 on its face. That Response noted that the specifics of the publication were being sought from counsel's associates in Japan, and that those specifics would be supplied on receipt.

It is first noted that the paragraph bridging pages 15 and 16 of the prior Response, which discussed Document BD, states that the article bears a statement that it was received for publication on "June 13, 1993". That date was an inadvertent error and should have been "June 13, 1990". Counsel regrets any inconvenience that error may have caused.

As to Document BD, Okamoto et al., Jpn. J. Exp. Med., 60(4):223-233 (1990), enclosed herewith as Exhibit I is a true copy of a fax received by counsel from Mr. Nobuo Ogawa of Nakamura & Partners, counsel's Japanese associate.

As will be seen from Exhibit I, there is a one-month disagreement between the date of mailing provided by the publisher and receipt date by libraries in the Tokyo area. Nevertheless, the earliest date, the receipt date, appears to be October 31, 1990, a date about two months after the filing date of this application. It is thus submitted that Document BD is not prior art here.

B. Further Art

Further art and an Action citing that art from co-assigned Application Serial No. 07/819,360 that also deals with HCV have been received. Non-redundant copies of that art (labeled Documents CA-CK) and the Action are included herewith, and that art is listed on enclosed Form PTO-1449.

No further fee or petition is believed necessary. However, should any further fee be needed, please charge our Deposit Account No. 04-1644, and deem this paper the required Petition.

Respectfully submitted,

By Edward P. Gamson
Edward P. Gamson, Reg. No. 29,381

Enclosures

Exhibit I
Action in Serial No. 07/819,360
Form PTO-1449
Art

CERTIFICATE OF MAILING

I hereby certify that this Amendment Under 37-C.F.R. §1.115, together with the stated enclosures, is being deposited with the United States Postal Service as First Class Mail, postage prepaid, in an envelope addressed to: Hon. Commissioner of Patents and Trademarks, Washington, D.C. 20231 on February 12, 1993.

Edward P. Gamson
Edward P. Gamson

NAKAMURA & PARTNERS
PATENT TRADEMARK & LEGAL AFFAIRS

Formerly
NAKAMATSU
 International Patent & Law Office

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 03-3214-6359 (G-II & G-III)
 CABLE: NAKAPATENT

EXHIBIT I

K. NAKAMATSU
 (1886-1973)

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 (ALPHABETICAL)

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 A. MAKODA
 T. NISHIMURA
 A. OSHIMA
 K. TSUJI

COUNSELLOR
 K. NISHIMOTO

February 4, 1993
 VIA FACSIMILE

Dressler, Goldsmith, Shore,
 Sutker & Milnamow, Ltd.
 Two Prudential Plaza
 Suite 4700
 Chicago, Illinois 60601
 U. S. A.

Attention: Mr. Edward P. Gamson

Re: OKamoto et al.
 Japanese Journal of Experimental Medicine
 60(4):223-233(1990)
 Your Ref.: NYBC 0025P and 0026P
 Our File: IPX-0011/MI/SEM

Dear Mr. Gamson:

Thank you for your letter of January 23, 1993 with respect to the above-identified journal published in Japan.

In accordance with your instructions, we have contacted KINOKUNIYA COMPANY LTD., the publisher of the subject journal. We talked to Mr. Masayuki FUNAMOTO of publication department about the mailing date of the subject journal, Vol. 60, No. 4, August 1990 issue, and learned that this journal was mailed out on November 28, 1990 according to the publisher's record. We also learned that this journal was actually printed and mailed out by subcontract printing company, CHUOH INSATSU JIMUKI, and that the publisher's record as to the mailing date was prepared based on the report from the subcontractor. We accordingly contacted the subcontractor and confirmed from a conversation with Mr. Chikara TAKIGUCHI that the

mailing date according to the subcontractor's record is exactly the same day, November 28, 1990. All of these conversation were made over the phone with Mrs. Setsuko MAYAMA, a patent attorney of our firm.

We, however, learned that the National Diet Library in Tokyo, one of the biggest comprehensive libraries in Japan, received the subject journal on October 31, 1990, one month before (not after!) the publisher's mailing date. The subject journal was sent to this library by mail and receiving date was indicated as a datemark on the front page of the journal. According to a library clerk of this library, the subject journal was probably available to the public a few days after the date of receiving, however, such date could not be identified since it is not a matter of record.

We also contacted departmental libraries of the University of Tokyo, i.e., the Medical Library and the Library of Faculty of Agriculture, and learned that the subject journals were independently sent by mail to these libraries on October 31 and November 1st, 1990, respectively. We were advised by a library clerk of the Medical Library that most journals and books are available to the university students on the very day or one day after the receiving date.

From the foregoing, we presume that the subject journal was received by libraries in Tokyo on October 31, 1990, at the earliest, and was available to the public at least by the end of the first week of November 1990, and that it would be most reasonable to consider that the subcontractor inadvertently recorded the incorrect mailing date and reported the incorrect date to the publisher. We again inquired the publisher and the subcontract printing company only to find that their records positively indicate the identical mailing date of November 28, 1990, approximately one month after the actual receiving date and that no other record was made by the subcontractor as to the mailing date that can be evidence to verify the actual mailing date.

We hope that you find the foregoing information sufficient for

your business purpose. If you have any questions or need further information, please do not hesitate to contact us again.

We will provide our debit note for services related to the above with the confirmation copy of this facsimile letter.

Very truly yours,

N. Ogawa

Nobuo OGAWA

MI/SEM/-



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07/819,360 01/10/92 KE

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DURRUE, C

DRESSLER, GOLDSMITH, SHORE, SUTKER &
MILNAROW, LTD.
TWO PRUDENTIAL PLAZA-SUITE 4700
180 NORTH STETSON AVENUE
CHICAGO, IL 60601

1813

DATE MAILED 01/13/93

☒ This application has been examined ☒ Responsive to communication filed on 10/28/12 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), — days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- | | |
|---|--|
| 1. <input checked="" type="checkbox"/> Notice of References Cited by Examiner, PTO-892. | 2. <input checked="" type="checkbox"/> Notice re Patent Drawing, PTO-945. |
| 3. <input type="checkbox"/> Notice of Art Cited by Applicant, PTO-1449. | 4. <input type="checkbox"/> Notice of Informal Patent Application, Form PTO-152. |
| 5. <input type="checkbox"/> Information on How to Effect Drawing Changes, PTO-1474. | 6. <input type="checkbox"/> |

Part II SUMMARY OF ACTION

1. ☒ Claims 1-25 are pending in the application.
Of the above, claims 10, 11, 15-25 are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 1-9 & 12-14 are rejected.
5. ☐ Claims _____ are objected to.
6. ☒ Claims 1-25 are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable, ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-945).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____, has (have) been ☐ approved by the examiner, ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved, ☐ disapproved (see explanation).
12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received, ☐ not been received.
☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

Serial Number: 07/819,360

-2-

Art Unit: 1813

15. This Application has previously been restricted under 35 U.S.C. § 121 (paper No. 7), and Applicants have responded in paper No. 8 to that requirement. Upon receiving the transferred case into Art Unit 1813, Examiner Dubrule determined that the prior requirement might be altered for clarity. The new Groups then are outlined below:

16. Restriction to one of the following inventions is required under 35 U.S.C. § 121:

I. Claims 1-9 and 12-14, drawn to peptides, immunogens and methods of immunizing, classified in Class 530, subclass 350 and Class 424, subclass 89.

II. Claims 10 and 11, drawn to antibodies, classified in Class 530, subclass 387.1.

III. Claims 15-21, drawn to assay methods for antibodies and kits, classified in Class 435, subclass 5.

IV. Claims 22-25, drawn to DNA molecules and assays using them, classified in Class 536, subclass 27 and Class 435 subclass 6.

17. The inventions are distinct, each from the other because of the following reasons:

18. The peptides of Group I could be used to generate the antibodies of Group II, or they could be used in antibody assay methods, as in Group III.

19. The antibodies of Group III could be used for diagnostic purposes (i.e., antigen capture), therapeutic purposes, or in purification schemes.

20. Group IV is distinct from the other Groups because DNA molecules are chemically distinct from protein molecules, and are useful for other purposes than encoding proteins, such as the assay of Group IV.

21. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification, restriction for examination purposes as indicated is proper.

22. During a telephone conversation with Edward Gamson on 1/7/93 a provisional election was made without traverse to prosecute the invention of Group I, claims 1-9 and 12-14. Affirmation of this election must be made by applicant in responding to this Office action. Claims 10, 11 and 15-25 are withdrawn from further consideration by the Examiner, 37 C.F.R. § 1.142(b), as being drawn to a non-elected invention.

Art Unit: 1813

23. The disclosure is objected to because of the following informalities: Page 73 of the specification reports at line 28 that 9 liters of RNase-free water were used to resuspend RNAs. The Examiner believes that this may be a typographical error. Page 80 of the specification refers to a Genebank Accession number, but fails to provide this number. The specification at page 81, line 21 refers to "the four major prototypes HCV-1, HCV-J and HCV-BK", which list includes only three major prototypes. Appropriate correction is required.

24. The following is a quotation of the first paragraph of 35 U.S.C. § 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

25. The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to adequately teach how to make and/or use the invention, i.e. failing to provide an enabling disclosure, and failing to adequately describe the invention.

26. The specification fails to teach Korean isolates of HCV. While the specification does characterize the Chinese isolates as belonging to the same group as Korean isolates, it is unclear how this conclusion was reached. The specification provides no sequence or homology data for Korean isolates, therefore claims directed to Korean isolates or sequences are not adequately described, in an enabling fashion.

27. The specification fails to teach "non-HCV-PRC-Korean unique" peptides or proteins, as recited in claim 6. As mentioned below, it is unclear what is meant by this phraseology, but for the purposes of this rejection the Examiner interprets it to mean "all peptides which are unique, but which are not derived from the PRC or Korean isolates". Clearly, the specification fails to teach such unique sequences. If Applicants intent was to claim non-unique portions of the PRC-Korean isolate, alternative language should be employed.

28. Claims 6, 7, 11 and 14 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

Serial Number: 07/819,360

-4-

Art Unit: 1813

29. Claims 6-9 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

30. As mentioned above, it is unclear what is meant by the term "a non-HCV-PRC-Korean unique". Perhaps affirmative language would be clearer.

31. Claims 12-14 are indefinite because it is unclear what is meant by the term "immunologically effective". Applicants do describe "effective amount" in the specification as that amount which can evoke an immune response (see page 48), but it is unclear if this defines the "immunologically effective" amount.

32. The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

33. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. § 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 C.F.R. § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. § 102(f) or (g) prior art under 35 U.S.C. § 103.

Art Unit: 1813

34. In order to clarify the record, the Examiner wishes to point out how the peptide claims are being interpreted. Claims to peptides, or which contain peptides within the claims are interpreted as follows:

A peptide having the amino acid sequence. - the peptide is the defined sequence, and does not include any other amino acids, i.e. NH_2 -(defined peptide)- COOH . This is equivalent to the judicially accepted term "consisting of".

A peptide which contains a peptide. - The peptide may include other amino acid residues, i.e. NH_2 -X-(defined peptide)-Y- COOH , where X and Y can be peptide or protein, or can be optionally not included. This is equivalent to the judicially accepted term "comprising".

35. Claim 1 is rejected under 35 U.S.C. § 103 as being unpatentable over Highfield et al, GB 2,239,245 in view of Lipka et al, 1990 and Washitani et al, 1991.

36. Highfield et al describe a novel isolate of HCV and its predicted amino acid sequence. One of the sequences (ID No. 3) contains the sequence of ID NO. 64 of the instant application (at residues 147-152). While the claim language of claim 1 excludes the intact protein represented by ID No. 3 of Highfield et al, the peptide is obvious from their disclosure. This is because the sequence of HCV is well known to vary greatly among isolates (see for example Ogata et al, 1991, Weiner et al, 1991, Choo et al, 1991 and Hijikata et al, 1991), therefore any portion of the sequence which is unique to an isolate or group of isolates would be useful to develop typing reagents, or to detect antibodies specific for that isolate. Both Lipka et al, 1990 and Washitani et al, 1991 teach the utility of such typing reagent to identify subtypes of viruses.

37. Claims 1-7 and 12-14 are rejected under 35 U.S.C. § 103 as being unpatentable over Highfield et al, Ogata et al, 1991, Weiner et al, 1991, Choo et al, 1991 and Hijikata et al, 1991, in view of Lipka et al and Washitani et al.

38. In each of the above cited primary references, standard cloning techniques were used to isolate and sequence various isolates and regions of HCV. Highfield et al teach the use of fusion proteins of HCV, as well as the use of antigenic regions to generate antibodies. A fair reading of the references teaches the heterogeneity of isolate sequences. In view of these references, the skilled artisan would have expected that novel isolates of HCV would have possessed unique regions.

Art Unit: 1813

39. The Examiner admits that the sequences represented in claims 1-6, with the exception of ID No. 64, fail to appear in the prior art made of record herein. Additionally, the skilled artisan would have been unable to predict what those sequences may have been based upon the prior art. However, it appears well known that the sequences of various isolates of HCV vary substantially (see above). Therefore, the fact that Applicants were able to isolate RNAs of HCV isolates which diverge from known isolates cannot be characterized as unexpected.

40. The motivation for identifying and producing peptides corresponding to unique regions would have been to develop typing reagents, not unlike those described by Lipka et al and Washitani et al.

41. In rejecting these claims, the Examiner is relying in no small part upon the decision the Board reached in Ex Parte Erlich, 22 USPQ 2d, 1463-1468. In this decision, the Board concluded that the specific hybridomas claimed were obvious over the prior art. Clearly, the specific cell lines claimed could not identically be reproduced by the skilled artisan, because of the complexity inherent to a composition such as a cell, but cell lines of similar function would have been producible.

42. While the specific sequences claimed instantly could not have been predicted based upon the prior art, the skilled artisan would have expected divergent sequences to exist in novel isolates.

43. Claim 8 is rejected under 35 U.S.C. § 103 as being unpatentable over Highfield et al, Ogata et al, 1991, Weiner et al, 1991, Choo et al, 1991 and Hijikata et al, 1991, in view of Lipka et al and Washitani et al as applied to claim 6 above, and further in view of Smith et al, 1988.

44. While Highfield et al employ fusion proteins in order to develop a more reliable assay, Smith et al teach that GST fusion proteins are easily purified. It would have been obvious to produce the expected peptides as fusion proteins on GST in order to facilitate downstream processing of the proteins (i.e. single step purification).

45. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

46. Papers related to this application may be submitted to Group 180 by facsimile transmission. papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989).

Serial Number: 07/819,360

-7-

Art Unit: 1813

The CM-1 Fax Center number is (703) 308-4227

47. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Chris Dubrule whose telephone number is (703) 308-0708. Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

CJD

GO

CHRISTINE M. NUCKER
SUPERVISORY PATENT EXAMINER
GROUP 180

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

FORM PTO-892 (REV. 2-82)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		SERIAL NO. 07819,160	GROUP/ART UNIT 1813	ATTACHMENT TO PAPER NUMBER 9		
NOTICE OF REFERENCES CITED				APPLICANT'S Wecta				
U.S. PATENT DOCUMENTS								
		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS		
A								
B								
C								
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F								
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FOREIGN PATENT DOCUMENTS								
		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SPTS. & PP. DWG. SPEC.
L								
M								
N								
O								
P								
Q								
OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)								
		Chao et al., PNAS 88:2451-2455, 1991.						
		A. Weiner et al., Virology 180: 842-848, 1991						
		Oyler et al., PNAS 88:3312-3316, 1991						
		Smith et al., GENET 67:21-40, 1988						
		Parker et al., J. Immun. 136(7): 2393-2397, 1986						
		Parker et al., J. Immun. 142(3): 971-976, 1989						
		Parker et al., J. Immun. 135(1): 247-254, 1985						
EXAMINER		This Dwyer						
		DATE 1/8/92						
* A copy of this reference is not being furnished with this office action. (See Manual of Patent Examining Procedure, section 707.05 (a).)								

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

FORM PTO-852 (REV. 2-92)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		SERIAL NO. 07819360	GROUP/PART UNIT 1413	ATTACHMENT TO PAGE NUMBER 9	
NOTICE OF REFERENCES CITED				APPLICANT(S) K et al			
U.S. PATENT DOCUMENTS							
*	DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE	
A							
B							
C							
D							
E							
F							
G							
H							
I							
J							
K							
FOREIGN PATENT DOCUMENTS							
*	DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS DWG SPEC.
L	ZZ3 9245	6/24/91	GB	Hughes et al			
M	1010231	9/7/90	NO	Blumberg et al			
N							
O							
P							
Q							
OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)							
R	Kipke et al, J. Int'l. Dis., 162:353-357, 1990						
S	Washburn et al, Int'l J. Pers., 49:173-177, 1991						
T	Kato et al, PNAS, 87:9524-9528, 1990						
U	Takamizawa et al, J. Virol., 65(3):1105-1113, 1991						
V	Okumoto et al, J. Exp. Med., 60(3):167-171, 1990						
W	Takahashi et al, JAR, 18(15):4626, 1990						
X	Harris et al, J. Immunol., 145(2):72-76, 1990						
Y	Hirakawa et al, BBRC, 175(1):220-228, 1991						
EXAMINER		DATE					
Christophe		1/8/12					
* A copy of this reference is not being furnished with this office action. (See Manual of Patent Examining Procedure, section 707.05 (a).)							

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Zabedee et al.)	Attorney Docket
Serial No.:	07/573,643)	PHA 0025P
Filed:	August 27, 1990)	Group Art Unit: 1802
For:	NON-A, NON-B, HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES)	
Examiner:	D. Wortman)	

SECOND DECLARATION OF ALFRED M. PRINCE, M.D.

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

*7/7/93
D.L.W.*
Sir:

ALFRED M. PRINCE, M.D., Declares

1. That he is the Alfred M. Prince who is a named co-inventor of the subject matter of the above-identified patent application and who submitted a Declaration mailed on January 28, 1993;
2. That the further data referred to in Paragraphs 6-10 in his previous Declaration were obtained using an ELISA format assay in which the recombinant 1-120 residue protein antigen was affixed to the walls of microtiter plates and used to assay for the presence of anti-HCV antibodies in sera of humans;
3. That enclosed Exhibit I is a true copy of the ELISA assay protocol used in his laboratory for carrying out ELISA assays using the recombinant 1-120 residue antigen and other recombinant antigens in similar assays;

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4. That the data of Paragraphs 6-10 of his previous Declaration, and particularly those of Table 1, using the C-100 antigen were also ELISA assays so that the data of Table 1 compare ELISA results to ELISA results;

5. That the data using the C-100 antigen ELISA were obtained following the procedures outlined in the manufacturer's (Ortho Diagnostics) instructions;

6. That the Nasoff et al. article (previously submitted Document BC) included data from assays using both immunoblot (Tables 1 and 2, page 5465) and ELISA (Table 3, page 5465) techniques;

7. That the data were taken for the recombinant 1-74 residue antigen (CAP-A) as discussed for immunoblots in Document BC, which method is substantially identical to the method discussed in the above-identified application, and that the ELISA data were taken as discussed in that paper, which method is substantially the same as that of enclosed Exhibit I;

8. That the data in Table 3 of Document BC show that ELISA data (asterisk) based on the recombinant 1-74 residue antigen (identified in Document BC as CAP-A) detected anti-HCV antibodies earlier than did the C-100 antigen-based ELISA in two out of three sera, and that immunoblots using the CAP-A antigen (dagger) detected infection earlier than did the C-100 antigen-based ELISA in four out of five sera;

9. That since the filing of his previous Declaration he has spoken with Dr. Chang Yi Wang about the identity of the

Serial No. 07/573,643

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synthetic core-structural (ST) peptide that was discussed in Paragraphs 20-24 of his previous Declaration;

10. That Dr. Wang is a co-author of Hosein et al., Proc. Natl. Acad. Sci. USA, 88:3647-3651 (1991), a true copy of which was enclosed as Document BA with the previous response, and the first-named inventor of U.S. Patent No. 5,106,726 of record herein;

11. That Dr. Wang fax'd a reply, a true copy of which is enclosed as Exhibit II;

12. That as is seen from enclosed Exhibit II, the core-structural peptide was actually a mixture of two peptides; i.e., VIIIE and IXD of U.S. Patent No. 5,106,726, so that the stated belief of previous Paragraph 20 was partially correct and the true identity of the UBI-ST antigen is now known;

13. That the data of Paragraphs 20-24 of his previous Declaration thus compared an ELISA assay based on the recombinant 1-120 residue protein (CAP) to an ELISA (UBI-ST) using the two overlapping peptides VIIIE and IXD that also encompass the 1-120 region;

14. That the CAP-based ELISA was also carried out following the protocols of enclosed Exhibit I, whereas the UBI-ST ELISA was carried out following the protocol supplied by UBI;

15. That it is now seen that the two antigens, both containing the same region, produced different results, with the recombinant antigen of the present claims providing a superior result;

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16. That the ELISA data reported in previously provided Document BB [Sugitani et al., The Lancet, 339:1018-1019 (1992)] were carried out for others' kits pursuant to the manufacturers' directions, and for the "Capsid" (recombinant 1-120 residue) antigen as discussed in Document BC; and

17. That he further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing on this application.

Date

April 20 1993Alfred M. Prince, M.D.

Enclosures

EXHIBIT I

HCV CORE ELISA

10-28-90 - Method #2

Materials:

1. Hepatitis C Capsid Protein (PHAGE Lot, # NB53P255) in 4M Urea. Source: E. coli (W3110/pGEX7 - Capsid #10)
2. Nunc Immuno MaxiSorp 96 well plates
3. Goat Serum, Gibco Labs
4. Bovine Serum Albumin - Fraction V, MW = 68,000, Boehringer Mannheim
5. 3M H2SO4
6. Sodium Carbonate buffer, pH 9.6 (coating buffer)
7. 0.1 M Sodium Citrate buffer, pH 5.0
8. OPD tablets, Zymed Labs.
9. HRP conjugated Anti Human IgG (Kirkegard & Perry)
10. PBS, pH 7.2
11. 5% Tween 20 in PBS

Procedure:

A. Coating of plates

1. Dilute the protein to 1 ug/ml in coating buffer containing 4M Urea:
For 20 mls of coating buffer - 26 ul of protein
4.2 g Urea
2. Add 100 ul to all wells
3. Cover and let stand overnight at RT
4. Refrigerate plates in a moist chamber and use within 1 week (longer storage will need to be tested in the future)

B. Assay

1. For each plate to be done prepare 40 mls of PBS containing 10% Goat sera (4mls) and 3% BSA (1.2g), and 0.05% Tween 20 (0.4 ml of 5% Tween 20).
This is the diluent for steps 3, 5, and 7
2. Wash plate 3X with PBS/Tween 20 (0.05%)
3. Add 150 ul of diluent to each well and block for 2 hrs 37 C. The plate can now remain at room temp until ready to proceed
4. Wash plate 3X with PBS/Tween
5. Prepare a 1:50 dilution of the sera with diluent and add 100 ul to wells. Include a positive and negative control in row 1 as follows:
Row A - Blank
Rows B-D - Neg cont diluted 1:50
Rows E-F - Pos cont diluted 1:50
Rows G-H - Pos cont diluted 1:500
6. Incubate 15 min 37 C
7. Wash plate 5 X, add 100 ul of conj currently used

Kirkegard and Perry 1:2000

8. Incubate 15 min 37 C

9. 10 minutes before incubation is over prepare OPD:

12 ml Citrate buffer

12 ul H2O2

1 OPD tablet

10. Wash plate 5 X, add 100 ul of OPD solution

11. Incubate 20 min RT in the dark.

12. Stop with 50 ul of 3M H2SO4 and read at T490/R630

13. CUTOFF:

Human Sera - Ave neg controls + 0.300

Chimp Sera - Ave neg controls + 0.200

HCV

EXHIBIT II



UNITED BIOMEDICAL, INC.
25 Davids Drive, Hauppauge, NY 11788 • (516) 273-2828 • Fax: (516) 273-1717

March 8, 1993

Dr. Alfred Prince
New York Blood Center
310 E. 67th Street
New York, NY 10021

Dear Fred:

As per our discussion yesterday, I am sending you a copy of our U.S. Patent #5,106,726 on HCV peptides.

The peptides used in our HCV core/at EIA are designated VIIIE & IXD as illustrated in Example 15. Their sequences can be found in claim 22.

I look forward to meeting you and your colleagues in mid-April for a fruitful scientific discussion.

Regards,

Chang Yi Wang, Ph.D.
Chief Scientific Officer

CYW:lx
Encl.

Applicant: Zebedee et al.

)
) Attorney Docket
) PHA 0025P

) Group Art Unit: 1802

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3

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

DR. TORSTEN B. HELTING , Declares,

1. That he was the President of Pharmacia Genetic Engineering Inc. (Phage) of La Jolla, California, an original co-assignee of the subject application;
2. That Phage, since the filing of this application, has been dissolved and its assets assumed by Pharmacia Biosystems, Inc., of Piscataway, New Jersey;
3. That as part of his duties at Phage, he entered into license negotiations with several potential licensees;
4. That as a result of his efforts, one license agreement has been signed with Sorin Biomedica of Italy and an option for a license has been signed with Behringwerke of Germany, both the license and option being active;

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5. That the license and option have related to the presently pending method claims and associated technology, and particularly the use of an ELISA format assay utilizing the recombinant 1-120 residue protein as antigen;

6. That the license and option have thus far generated several hundred thousand dollars in fees;

7. That he is in the midst of further negotiations with other potential licensees who are presently evaluating an ELISA kit based on the recombinant 1-120 residue antigen with those evaluations not yet completed; and

8. That he further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing on this application.

April 20, 1993
Date


Dr. Torsten B. Helting

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee et al.)
Serial No.: 07/573,643) Attorney Docket
Filed: August 27, 1990) PHA 0025P
For: NON-A, NON-B, HEPATITIS VIRUS)
ANTIGEN, DIAGNOSTIC METHODS AND)
VACCINES) Group Art Unit: 1802
Examiner: D. Wortman)

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LP
C-2793

RECEIVED
APR 27 1993
GROUP 1800

SECOND SUPPLEMENTAL RESPONSE

Box Non-Fee Amendments (Pats)
Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

This is in further response to the Office Action dated October 5, 1993 to which a previous response was filed on January 28, 1993, a Supplemental Response was filed on February 12, 1993, and a personal interview was held with the Examiner on February 23, 1993.

RESPONSE

Reconsideration of the above-identified application is respectfully requested in view of the previously filed Response, Supplemental Response and the present, Second Supplemental Response.

The Examiner is thanked for the courtesies extended and comments made to Dr. Helting and counsel during their interview. This Response will address those comments.

Claims 28-37 are before the Examiner.

Inasmuch as there was no discussion during the interview of the previously outstanding rejection under 35 U.S.C. §112, second paragraph, that was responded to in the Response mailed January 28, 1993, no discussion will be had here on that issue. Rather, the present response will deal with the alleged anticipation/obviousness issues and the previously filed Declaration of Dr. Prince.

A. The Second Prince Declaration

Dr. Prince's enclosed, Second Declaration points out (Paragraphs 2-5) that the data of Paragraphs 6-10 of his previous Declaration were taken by ELISA. The procedures provided with the commercial kit (C-100 antigen) were used, and a protocol for the ELISA using a recombinant of the claims is enclosed therewith. Thus, the data of Dr. Prince's previous Paragraphs 6-10 compared ELISA to ELISA, and showed an ELISA based upon a claimed recombinant to outperform the then industry standard, commercial C-100 antigen-based ELISA.

Paragraphs 6-8 of Dr. Prince's present Declaration point out that ELISA and immunoblot assays were also carried out as discussed in previously submitted Document BC using a recombinant 1-74 residue antigen of the claims, with the details for each of those assays being given in Document BC. Present Paragraph 8 points to Table 3 of Document BC in showing that an ELISA based on that recombinant 1-74 residue antigen also outperformed the industry standard C-100 antigen-based ELISA

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-3-

assay for two of three sera, whereas an immunoblot based on that same recombinant outperformed the C-100 antigen-based ELISA with four of five sera.

Paragraphs 9-15 of Dr. Prince's present Declaration discuss the identity of the antigen of the UBI ELISA kit he obtained denominated "ST" and the data obtained therewith, as compared to an ELISA based on a recombinant 1-120 residue antigen of the claims. The results of those ELISA assays were discussed in Paragraphs 20-24 of Dr. Prince's prior Declaration. The present Declaration also discusses how those ELISA assays were done.

As is seen from Dr. Prince's present Paragraphs 9-15 and Exhibit II enclosed therewith, Dr. Wang has now identified the peptide antigen used in the "ST" ELISA as being both of peptides VIIIE and IXD of her U.S. Patent No. 5,106,726. Thus, the results discussed in the previous Prince Declaration Paragraphs 20-24 compared ELISA to ELISA with a recombinant antigen and synthetic peptides covering the exact same region. Those results also showed a method using a claimed recombinant antigen to be superior.

Paragraph 16 of Dr. Prince's present Declaration discussed how the ELISA's using others' kits and an ELISA based on the recombinant 1-120 residue antigen of previously submitted Document BB were carried out.

The immunoblot data of the application and the ELISA and immunoblot data of Dr. Prince's previous and present Declarations show the following:

(a) a method using immunoblots with a recombinant 1-74 residue antigen detected HCV infection earlier than did an ELISA assay based on the C-100 non-structural antigen (application Tables 2, 3, 4, 5 and 6; 2nd Declaration Par. 6-8; Document BC, Table 3, dagger data);

(b) ELISA comparisons between a recombinant antigen having the 1-74 residue sequence and C-100 showed the method using the 1-74 residue recombinant of the claims to be superior (Document BC, Table 3, asterisk data);

(c) ELISA method comparisons using the C-100 commercial antigen and the 1-120 residue recombinant of the claims showed the claimed antigen to be superior (1st Declaration Par. 6-10; 2nd Declaration Par. 2-5);

(d) ELISA method comparisons using Dr. Wang's synthetic peptides VIIIE and IXD ("ST" kit) and a recombinant 1-120 residue of the claims as antigens showed a claimed method using a recombinant antigen to be superior to use of synthetic peptides covering the same region as antigen (1st Declaration Par. 20-24; 2nd Declaration Par. 9-15); and

(e) ELISA method data using a recombinant 1-120 residue antigen of the present claims provided the same results as an ELISA method containing synthetic peptides from both

structural and non-structural proteins (UBI-HCV) (1st Declaration Par. 16-19; 2nd Declaration Par. 16; Document BB).

Thus, the data provided show a claimed assay method using a recited recombinant antigen to be: (a) superior to the C-100 antigen based ELISA by ELISA and immunoblot, (b) superior to an antigen containing a combination of synthetic peptides covering the same region by ELISA, and (c) comparable to an ELISA using synthetic antigens from both structural and non-structural regions.

B. The Helting Declaration

Doctor Helting's enclosed Declaration is directed to the commercial success thus far achieved by a claimed method of the invention that utilizes a recombinant 1-120 antigen, and that is practiced in commercial form via a kit using that recombinant as the solid phase antigen.

Dr. Helting's Declaration points out that even though the original assignee, Pharmacia Genetic Engineering, Inc. (Phage), has been absorbed into another entity, he was successful in negotiating an active license and an active option for a license to practice a claimed method via a kit. His Declaration also notes that those separate license and option agreements have generated several hundred thousand dollars in fees. He further notes that still other potential licensees are evaluating an assay kit that utilizes a claimed method, with the results of those evaluations still not in hand.

which one?

C. The Art-Based Rejections

Each of the art-based rejections was tied to the disclosures of the Wang et al. U.S. Patent No. 5,106,726, hereinafter referred to as "Wang", and each of those rejections was dealt with in the prior response. This response will therefore be limited to the unexpected results discussed before and to the commercial success thus far achieved.

The Response mailed January 28, 1992 (the previous Response) noted that the data of the Wang disclosure compared assay results using an ELISA format with a synthetic peptide as antigen. Those results were compared to Wang's synthetic peptide IIID, whose ELISA results were compared with an ELISA based on the C-100 antigen.

When ELISA results for peptides VIIIE and IXD were compared to those for peptide IIID (Table 7), and thus to an ELISA with C-100, the best that was observed was a result that was 98.6 percent as good as a C-100 antigen-based assay. As pointed out above and in Dr. Prince's Declarations, ELISA assays based on a claimed method using a recombinant antigen outperformed the C-100 antigen-based assay.

Thus, both the Wang data and the Prince Declaration data were compared to the same standard with the same basic method steps but different antigens. The Wang binding method data underperformed the standard, whereas the inventors' method using a recombinant antigen outperformed that standard.

The data of the application also show that an immunoblot method of the claims using a recombinant antigen also outperformed a C-100 antigen-based assay.

The data and discussions of Dr. Prince's Declarations also show that a claimed method using an ELISA format with recombinant 1-120 residue antigen outperformed an ELISA using mixed Wang peptides VIIIE and IXD as antigen. The two antigens of those head-to-head ELISA's covered the same region, and yet a claimed method using a recombinant antigen outperformed a similar method using mixed Wang peptides.

The data and discussions of both Prince Declarations also point out that a method using an ELISA format with a single recombinant 1-120 residue antigen provided the same results as a method using an ELISA format and Wang synthetic peptides from both structural and non-structural regions. Thus, a claimed method using a single recombinant antigen from a single protein provided the same results as a similar method using multiple antigens encoded by a plurality of regions of the genome.

It is thus submitted that the disclosures of the application and data and discussions of Dr. Prince's Declarations utilizing claimed methods provide unexpected and unobvious results compared to the teachings of Wang.

Dr. Helting's Declaration also provides additional indicia of unobviousness. His achievements in obtaining a license and an option for a license have already earned several hundred thousand dollars in fees for the claimed technology.

It must be understood that the diagnostic niche for HCV has largely been filled by the Chiron-Ortho and Abbott (Abbott is a licensee of Ortho) C-100 antigen-based kit and the second generation kits as noted in previously submitted Document BB. A kit whose use embodies a claimed method is thus a newcomer to the field.

A newcomer must have something extra to offer a potential licensee. It is submitted that that something extra must include equal or better efficacy or the licensee /optionee will not be able to sell the product.

The ELISA data of Document BB illustrate that a kit whose use entails a claimed method (Capsid) with the single recombinant 1-120 residue antigen provided equivalent efficacy to the second generation kit marketed by Abbott (Abbott II) and the UBI HCV kit that contain antigens from two regions. As noted before, that a single antigen-based method would provide such efficacious results was unexpected. That unexpected result also provided the something extra that gave a basis for the licensee and optionee to pay hundreds of thousands of dollars in fees.

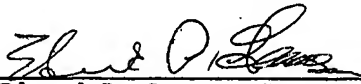
It is again submitted that the invention claimed here is not anticipated or obvious from Wang's disclosures alone or used in combination with any other teaching of record. It is thus submitted that the presently claimed subject matter is deserving of a patent in view of its unexpected and unobvious character.

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No further fee or petition is believed necessary.
However, should any further fee be needed, please charge our
Deposit Account No. 04-1644, and deem this paper the required
Petition.

Respectfully submitted,

By 
Edward P. Gamson, Reg. No. 29,381

Enclosures

Second Declaration of Dr. Alfred M. Prince, M.D.
Declaration of Dr. Torsten B. Helting



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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07/573,643 08/27/90 ZEBEDEE

18M1/0713

DRESSLER, GOLDSMITH, SHORE,
SUTKER & MILNAMOW, LTD.
11300 SORRENTO VALLEY RD., STE. 200
SAN DIEGO, CA 92121

S PHA0025P

EXAMINER

WORTMAN, D.

ART UNIT

PAPER NUMBER

22

1802

DATE MAILED:

07/13/93

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 2/1/93 2/16/93 4/27/93 ☒ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- | | |
|---|---|
| 1. <input type="checkbox"/> Notice of References Cited by Examiner, PTO-892. | 2. <input type="checkbox"/> Notice of Draftsman's Patent Drawing Review, PTO-948. |
| 3. <input checked="" type="checkbox"/> Notice of Art Cited by Applicant, PTO-1449. | 4. <input type="checkbox"/> Notice of Informal Patent Application, PTO-152. |
| 5. <input type="checkbox"/> Information on How to Effect Drawing Changes, PTO-1474. | 6. <input type="checkbox"/> _____ |

Part II SUMMARY OF ACTION

1. ☒ Claims 28-87 are pending in the application.

Of the above, claims _____ are withdrawn from consideration.

2. ☒ Claims 1-27, 38-40 have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☒ Claims 28-37 are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☐ This application has been filed with Informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8. ☐ Formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____ Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).

10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____, has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).

12. ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.

13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☐ Other

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Claims 28-37 are currently under examination. Claims 28, 29, and 30 were amended in Paper No. 16.

Claims 28-37 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 28 is indefinite because it is unclear whether "including an amino acid sequence" is intended to encompass only sequences with the same specific start and end points as recited or every protein or polypeptide with any amino acid sequence in common with those specifically recited.

Applicant has urged that "including" is synonymous with "comprising" and that the present claims should be understood to encompass any recombinant NANBV structural protein or portion that has an enumerated sequence from residue 1 through residue 74 or the entire sequence from residue 1 through residue 120.

This argument is not completely understood because it is not clear what it means to say that a "protein or portion has an enumerated sequence" (underlining added), i.e. it is not clear whether the protein has exactly the same number of amino acids recited or whether it may have additional amino acids as well.

In addition, Applicant has submitted a copy of pp. I-10.1 through I-13 from Aisenberg's Attorney's Dictionary of Patent Claims showing patent claims that include "including." In that regard, it is noted that, on page I-10.1, the footnote reads in part "Although this term is used in claims of a number of issued patents, it may be regarded as introducing an element of

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indefiniteness. A better view appears to be that the "including" phrase adds further, although not exclusive, definition."

Whether "including" is equivalent to "comprising" or introduces an element of indefiniteness or adds further but not exclusive definition, its use makes the claims indefinite because one cannot readily determine what is being claimed. The rejection of Claims 28-37 under 35 U.S.C. § 112, second paragraph, is maintained.

Applicant has submitted five articles as well as a form PTO 1449, recorded as Paper No. 18, on which those articles are listed. In addition, Applicant has submitted another PTO 1449 with references attached to the Supplemental Response which is recorded as Paper No. 20. All of these references have been placed in the file and considered to the extent they bear on Applicant's remarks but have been crossed off the forms 1449 because neither information disclosure statement complies with 37 CFR 1.97(c).

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. § 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 C.F.R. § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. § 102(f) or (g) prior art under 35 U.S.C. § 103.

Claims 28-30 as amended and 31-37 are rejected under 35 U.S.C. § 103 as obvious over the patent to Wang in view of Kuo. Wang teaches assaying sera for antibodies against HCV (NANBV) using solid phase coated with synthetic peptides that include amino acid residue sequences as instantly claimed (see Wang,

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Example 14 and Table 7, especially peptides VIIIE, IXD, and IXE).

It is noted that "including" as recited in Claim 28 encompasses any common amino acid sequence. Even if the instant sequences were recited more narrowly, they would have been obvious over Wang because there are only slight differences between them and the core sequences taught by Wang. Wang does not teach producing recombinant HCV polypeptides. Kuo teaches production of an HCV recombinant fusion protein and detection of serum antibodies using the recombinant fusion protein. It would have been obvious to one of ordinary skill in the art to produce the HCV peptide of Wang recombinantly as taught by Kuo in order to gain the advantages of producing peptides by recombinant means, e.g., to obtain a stable, plentiful supply of peptides that are free of contamination with other HCV antigens.

Applicant has submitted Papers 16, 20, and 21 in response to the last Office action and also two Declarations from Dr. Prince and a Declaration from Dr. Helting.

Applicant has argued in Paper No. 16 that:

- 1) Wang's antigens are all synthetic antigens and the instant claims are now limited to recombinant antigens.
- 2) Applicant's results using capsid amino acids 1-74 as a GST fusion protein are unexpectedly superior to Wang's results and in particular, points to various results of Wang, and compares the instant invention with results obtained using C-100, using as supporting documents references which were supplied by Applicant.

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Applicant has submitted declarations by Dr. Prince referring to supporting documents comparing Wang's synthetic peptides with applicant's fusion protein containing HCV capsid amino acids 1-74.

- 5 3) Kuo's recombinant antigens are produced in yeast and the instant polypeptides are produced in E. coli.
- 4) Applicant's results using capsid amino acids 1-120 (apparently not as a fusion protein) are unexpectedly better than Wang's and commercial success has been achieved for a test using that
- 10 composition. Applicant has submitted Declarations from Dr. Prince in support of unexpected results and a Declaration from Dr. Helting in support of claims of unexpected results and commercial success.

These arguments and documents have all been considered but

15 not found persuasive for the following reasons.

- 1) Wang does not use recombinant antigen but Kuo does; Kuo was cited to show recombinantly produced HCV peptides.
- 2) All comparisons of the sequence 1-74 to prior art antigens were apparently done using GST fusion protein and in immunoblot
- 20 format and not all the claims are so limited. In addition, the results of Cap-N as compared to the results of Wang using VIIIE do not appear to be unexpectedly different, taking into account the teachings of Wang: the same sequence in two different environments can produce different and unpredictable antibody
- 25 binding results. Furthermore, with respect to epitopes located on

HCV core antigen Wang teaches that her results indicate a complicated epitope distribution and may include discontinuous or conformational epitopes (col.41, line 66-col. 42, line 3). Wang provides motivation to use core peptides rather than

5 nonstructural peptides like C100 because Wang shows that assays using core peptides detect antibodies earlier in the infection (Wang, Table 8). With respect to the citation of document BC, one would have expected 69-120 to be less effective in binding antibodies than a peptide from the amino terminus based on Wang's
10 results showing more epitopes on the amino terminus.

3) The instant claims are not limited to recombinant antigen expressed in E. coli.

4) Neither the arguments presented nor the Declarations of Dr. Prince and Dr. Helting are commensurate in scope with the claims
15 because the Declarations of Dr. Prince only concern the comparison of results obtained with a fusion protein GST-amino acid 1-74 and with capsid protein 1-120 produced recombinantly in E. coli to results obtained with C-100 and Wang's capsid peptides. The second declaration of Dr. Prince, page 2,

20 paragraph 8, states that the "CAP-A" antigen (which contains capsid amino acids 1-74) detected HCV antibodies earlier in infection than did the C-100 antigen. However, one would have expected that from Wang because Wang also shows that test compositions including only core or capsid sequences or those
25 containing core + ns antigen detected antibodies sooner than

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compositions containing only ns sequences (See, for example, Wang, Table 8). The declaration at page 3, paragraphs 13-15 refers to the results in Dr. Prince's first declaration, paragraphs 20-24, comparing the instant recombinant 1-120 antigen
5 with Wang's two synthetic peptides covering most of the same sequence as showing a superior result. However, these results are not seen as being totally unexpected because Wang teaches that the same sequence in two different environments can produce different antibody binding results. Furthermore, with respect to
10 epitopes located on HCV core antigen Wang teaches that her results indicate a complicated epitope distribution and may include discontinuous or conformational epitopes (col.41, line 66-col. 42, line 3). In fact, the comparison of the two antigens shows similar results are obtained in most cases (Dr. Prince's
15 first Declaration, paragraph 21: "That the results for those sera were comparable with the exception of the sera from a chimpanzee designated Chimpanzee No. 10³" and in only the one case, the instant antigen was able to detect antibodies when Dr. Wang's peptide mixture did not. One might have expected the
20 intact 1-120 sequence to contain one or more discontinuous or conformational epitopes that would be disrupted if the sequence were presented as two peptides, based on Wang's teachings as discussed above.

Dr. Helting's Declaration has been considered but is not
25 found to be sufficient evidence of commercial success to make the

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instant invention unobvious over prior art of record. There is no information, for example, as to the terms of the license and option agreements mentioned. There is no information as to whether other, competing products are readily available in the same markets for the same price. Nothing can be concluded from evaluations not yet completed. In addition, Dr. Helting's Declaration is not commensurate in scope with the claims since it only concerns recombinant 1-120 antigen.

Applicant's amendment necessitated the new grounds of rejection. Accordingly, THIS ACTION IS MADE FINAL. See M.P.E.P. § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. § 1.136(a).

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 C.F.R. § 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform to the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 308-4227.

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Any inquiry concerning this communication should be directed
to Examiner Donna C. Wortman at telephone number (703) 308-1032.

Dw
Donna C. Wortman, Ph.D.
July 9, 1993

Esther Kepplinger

ESTHER L. KEPPLINGER
SUPERVISORY PATENT EXAMINER
GROUP ART UNIT 1802

200-126 CP 23
1802 10-21-43



BOX AF

RESPONSE UNDER 37 C.F.R. 116
EXPEDITED PROCEDURE
EXAMINING GROUP 1802

THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee et al.)
Serial No.: 07/573,643) Attorney Docket
Filed: August 27, 1990) PHA 0025P
For: NON-A, NON-B, HEPATITIS VIRUS)
ANTIGEN, DIAGNOSTIC METHODS AND)
VACCINES)
Examiner: D. Wortman)

AMENDMENT UNDER 37 C.F.R. §1.116

Box AF
Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

In response to the Office Action dated July 13, 1993,
please amend the above-identified application as follows.

IN THE CLAIMS

Please cancel claim 29.

Please amend claim 28 as follows:

28. (Three-Times Amended) A method of assaying a body
fluid sample for the presence of antibodies against NANBV, which
method comprises:

a) forming an immunoreaction admixture by
admixing said body fluid sample with a recombinant NANBV
structural protein or portion thereof, said recombinant protein
or portion [including an] having the amino acid residue sequence
represented by the sequence shown in [figure] Figure 1 from
residue 1 [to] through residue [74] 120 or fusion protein
sequence shown in Figure 2 from residue 1 [to] through residue
[120] 315;

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C1 b) maintaining said immunoreaction admixture for a time period sufficient for any of said antibodies present to immunoreact with said recombinant NANBV structural protein or portion to form an immunoreaction product;

and

c) detecting the presence of any of said immunoreaction product from and thereby the presence of said antibodies.

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the discussion that follows is respectfully requested.

Claim 28 has been amended, and claim 29 cancelled as discussed below. Claims 28 and 30-37 are before the Examiner.

I. The Amendments

Claim 28 has been amended to recite only the particular recombinantly produced NANBV protein or fusion protein-containing portion for which data have been provided. One is a fusion protein and the other is the substantially intact, isolated recombinant protein. The objected to words "including an" have been replaced with the phrase "having the" as to the sequence utilized.

Support for the present claim language can be found throughout the specification. The Examiner's attention is also specifically invited to page 11, lines 20-25; page 26, line 24 through page 28, line 10, and particularly to page 27, lines 11-20, as well as to page 57, lines 21-30 wherein the CAP-N fusion protein of the present claims is stated as having the sequence of Figure 2 from residue 1 to residue 315.

It is also noted that the syntax of claim 28 has been changed slightly so that there is a parallel construction between

the "protein" and the Figure 1, 1-120 sequence, and another parallel construction between "portion" and the sequence of Figure 2 at positions 1-315. Although no issue of a lack of clarity was raised in the Action, it is believed that the present language makes the claimed subject matter still clearer.

It is thus seen that no new matter has been added to the claims.

II. THE ACTION

A. Rejection Under 35 U.S.C. §112, Second Paragraph

The pending claims were rejected as being allegedly indefinite regarding the use of "including an amino acid [residue] sequence". It is believed that the present amendments that utilize the word "having" along with the word "or" removes the alleged indefiniteness and overcomes or makes moot this rejection.

B. Compliance with 37 C.F.R. §1.97(c)

The fee required under §1.97(c) is enclosed pursuant to the above section and §1.17(p). It is noted that the last paragraph of the two Supplemental Responses requested that any further fee be charged to counsel's Deposit Account. It is thus believed that the requirements of Section 1.97(c) have been met.

C. Rejection Under 35 U.S.C. §103

The pending claims were rejected as allegedly obvious over the disclosures of Wang in view of Kuo, both of which have been discussed several times in this prosecution. This rejection is respectfully traversed.

The Action asserts that Wang teaches use of synthetic HCV "peptides that include amino acid residue sequences as instantly claimed..." as antigens for solid phase assays. Actually, at column 29, lines 32-37, Wang teaches her peptides to be useful in not only solid phase assays, but also in an "enzyme

immunodot assay, an agglutination based assay, or other well-known immunoassay devices." The Action asserts the prior use of "including" encompasses any overlapping sequences and that narrowed sequences would still be obvious from Wang. The Action states that Wang does not teach use of recombinant peptides, but that Kuo teaches use of recombinant technology with other HCV proteins so that it would have been obvious to use Kuo's technique to make a Wang peptide.

The paragraph bridging Wang's columns 23 and 24 teaches several advantages and distinctions of her synthetic peptides over "biologic" materials for use in the assays contemplated. Among those advantages are the high yield, gram quantity amounts of synthetic peptide that can be obtained by which

"a reproducible antigen of high integrity with consistent yields [can be produced]. The presence of other antigens from biological systems precludes such reproducibility." [Column 23, lines 56-61.]

Those are contrarily some of the same attributes ascribed by the Action herein for recombinants. Wang's same paragraph continues by saying that even

"more importantly, non-specific reactivities seen in uninfected individuals are likely to be due to the heterogeneity of the preparations used for assay. This is particularly true for assays using biologically based immunoadsorbents." [Column 23, lines 61-65.]

The remainder of that paragraph discusses biologically based materials isolated from hosts. However, at column 5, lines 29-55, Wang also touts how wonderful synthetic peptides are for assays, citing to her U.S. Patents No. 4,735,896 and No. 4,879,212, and asserting "superior sensitivity, excellent specificity, ..." and viral differentiability for such materials, "thus overcoming many of the existing problems with biologically-

derived tests based on either viral lysate or recombinant DNA-derived protein." (Column 5, lines 49-55; emphasis supplied.)

It should thus be clear that recombinants as claimed here are among the "biologically based immunosorbants" whose use Wang's synthetic peptides were designed to replace.

As pointed out in re Fine, 5 USPQ2d 1596, 1499 (Fed.Cir. 1988), one tests obviousness by what the combined teachings of the references would have suggested to those of ordinary skill in the art. Obviousness cannot be established by combining teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. See, also, In re Jones, 21 USPQ2d 1941, 1944 (Fed.Cir. 1992).

Here, Wang teaches that one should not use biologically based antigens for several reasons, noted before. That is a teaching contrary to the Action's position that is from the very same art relied-on for this rejection. The combination is therefore improper, and this rejection should be withdrawn.

The Kuo teaching utilizes recombinant technology with a group of HCV proteins different from those claimed here. It has no teaching as to the present structural proteins, nor as to what primers or other materials to use to obtain a presently claimed recombinant antigen. Kuo also therefore teaches away from the Action's combination.

Thus, there is motivation in the relied-on art against making the Action's combination, and there is no teaching in either document as to how to make that which is claimed. The present basis for rejection is thus submitted to be a hindsight reconstruction, and should be withdrawn.

A similar situation arose in the recently published decision in In re Bell, 26 USPQ2d 1529 (Fed.Cir. 1993). There, a

DNA sequence of a known protein was claimed. One relied-on reference taught the protein sequence, and the other taught a general method of gene cloning. The Examiner and Board held the claimed DNA to be obvious. The Court disagreed.

Relying on Fine, supra, and similar cases, the Court held that the gene cloning reference neither taught nor "fairly suggested the combination of teachings "since it nowhere suggested how to apply its teachings ..." to other systems, there, "amino acid sequences without unique codons". (26 USPQ2d at 1532.)

The same reasoning should hold here. Kuo has no teaching as to how to apply its method to another HCV protein. Again, this rejection should be withdrawn.

In responding to the points made in the last responses, it is submitted that the Action, beginning near the bottom of page 6, has relied upon several misconceptions. Those will be dealt with below, in the order raised.

1. The inapplicability of the Wang and Kuo combination has been dealt with above.

2. The work discussed with the 1-74 recombinant was with the CAP-N fusion protein, and that is presently claimed. However, the data comparing that CAP-N fusion protein were obtained both by immunoblot (as disclosed generally by Wang) and by ELISA as were noted in Table 3 of document BC discussed in Dr. Prince's Declarations and near the bottom of page 2 of the Second Supplemental Response.

Thus, limitation of the claims to immunoblots should not be required. The 1-74 recombinant fusion protein outperformed C-100 in both types of assay.

As to differences between ELISA and immunoblot assays, ELISA's are often more sensitive, but less specific. That is the

reason Western blots are used to confirm HIV ELISA assays, as is well known. Regardless of which format is chosen, the steps are the same; i.e., formation of an immunocomplex and detection of same, and the results here showed that either presently claimed recombinant antigen outperformed the C-100 antigen in both ELISA and immunoblot assays both for sensitivity and specificity, whereas the data for Wang's Table 7 show her peptides to be at best only 98 percent as sensitive as C-100 in an ELISA. Thus, an unexpected result was shown here as Wang teaches that her synthetics are both more sensitive and specific than are biologically-derived antigens.

There is also a misconception as to Wang's teachings of "different and unpredictable antibody binding results" depending upon the environment. This misconception was possibly enhanced by counsel, for which he apologizes.

Wang has no specific teaching that different environments for an antigen can provide different and unpredictable results. Rather, her data show that fact, and that showing was referred to as a "teaching" by counsel at page 5 of the Response filed on January 28, 1993.

The purpose of that discussion was to point out simply that antibody binding results are not necessarily predictable and obvious simply because a given stretch of protein sequence is present, as the prior and present Actions would imply. Thus, Wang's results showed that merely having a sequence present was not sufficient for obtaining a useful result.

It is submitted that Wang's internally inconsistent results belie her broad assertions to superior sensitivity and excellent specificity for synthetic peptides. Wang's results must therefore be narrowly construed and limited only to the specific peptides she found useful.

The data of the present application and associated documents show the immunodominant region of the HCV capsid to be within the 1-74 region, and is centered near residues 21-40 as discussed hereinafter. The fusion protein partners for the claimed 1-74 and the discussed 21-40 protein portions and the C-terminal portion of claimed 1-120 recombinant provide carriers that optimally present that immunodominant domain to the assayed antibodies.

The Wang synthetic peptides fail to identify that region of immunodominance and are remarkable for their inability to outperform the C-100 antigen, which itself is not a particularly good antigen. The data of Table 7 show all of the Wang peptides to be poorer antigens than C-100.

The data of Wang's Figs. 11-1 and 11-2 actually show equivocal data. One serum (#4) was a uniformly poor binder; one serum (#1) bound well throughout the region; one (#2) bound well to all but the C-terminal region; and the last (#3) only bound near the N-terminus. The identification of which serum sample reacted with which peptide at Wang's column 41 does not agree with the results shown in those figures. Wang's presently relied-on statement as to discontinuous or conformational epitopes is thus seen to itself be a hindsight reconstruction to rationalize her poor results, and the failure of her touted concept of the use of peptides for "site-directed serology" (column 5, lines 29-33).

The results discussed during this prosecution regarding recombinant 1-74 fusion protein and a recombinant 21-40 fusion protein discussed hereinafter, as well as in co-pending Serial No. 07/616,369 that is also before the Examiner, and in Dr. Helting's present Declaration show that those two fusion proteins are similar to the recombinant 1-120 protein as antigens

in a contemplated assay, and each of the three outperformed the C-100 antigen. The immunoassay data of Table 1 of document BC using the recombinant 69-120 fusion protein (CAP-B) showed substantially no binding. Thus, there is little likelihood of there being a discontinuous linear or conformational epitope at capsid position 74-120 that is of significance in this type of immunoassay.

Indeed, Dr. Helting's enclosed Declaration states that he is unaware of the presence of such an epitope or any other epitope of significance in an immunoassay such as claimed here in the region between residues 74 and 120 at the C-terminus. It is also Dr. Helting's view that the C-terminal binding results reported in the Wang patent are the result of a generally low level of binding obtained with synthetic peptides disclosed by Wang, leading to observed binding by epitopes that do not contribute significantly to the performance of a recombinant-based immunoassay.

It also cannot be agreed that Wang provides motivation to use core rather than peptides from the non-structural C-100 region to provide earlier detection of antibodies. Rather, her data provide motivation to use core (capsid) plus such C-100-derived peptides. This point is underscored by the statements of the paragraph bridging pages 3650 and 3651 of document BA, and those at column 43, line 29 of the patent: "makes Format C an ideal candidate for a HCV screening assay". Format C contained two C-100 peptides (II H and V) and capsid peptide VIII E.

Thus, the data in Wang's Table 8 show that the C-100 plus core peptides provided an enhanced result over core alone in seven out of ten assays. The data of Table 1 of document BA by Wang and others provides different numbers for the same samples

(as noted before), but those different numbers follow the same trend.

The data provided herein show that the results obtained with the recombinant 1-120 were the same as those of Wang's C-100 plus core. (Prince Declaration, paragraph 19). In addition, that recombinant also outperformed Wang's two peptide mixture (VIIIIE and IXD) as to Chimp 10. As the Court pointed out in re Chupp, 2 USPQ2d 1437, 1439 (Fed.Cir. 1987), "[e]vidence that a compound is unexpectedly superior in one of a spectrum of common properties, as here, can be enough to rebut a prime facie case of obviousness" (citation omitted). Thus, the data as to Chimp 10 cannot be discounted.

3. It is agreed that the claims are not limited to recombinants produced in E. coli.

4. It is believed that the scope of the Declarations of Drs. Prince and Helting as to the recombinant 1-74 fusion protein and recombinant 1-120 protein are commensurate with the claims.

The Action's argument that the results of Dr. Prince's Declaration paragraphs 20-24 are not unexpected cannot be agreed with. The Action now asserts, as did a previous response, that Wang's data for a given sequence show unpredictability. That unpredictability is then used to show that a now admittedly superior result using a claimed recombinant is predictable and therefore obvious. It is submitted that an obvious result and an unpredictable result are antithetical.

The Action's failure to find Dr. Helting's Declaration concerning his licensing activities to contain sufficient evidence cannot be agreed with, nor can the additional questions that were raised be agreed to have been proper.

First, Dr. Helting's Declaration provided evidence that a licensee and an optionee had spent their money to obtain rights to an assay that presently is not protected by any patent. The amount spent by those third parties was noted to be several hundred thousand dollars.

As the Examiner should know, price information is a guarded secret among licensees and licensors. As a consequence, that information is not available to third parties, such as Dr. Helting or applicants.

Dr. Helting's enclosed Second Declaration in response to the points raised in this Action notes his understanding that data on the UBI technology were assembled and evaluated by both Sorin Biomedica and Behringwerke, the present licensee and optionee of a method of the present claims. The UBI technology and that utilizing a claimed invention were assessed for their performance prior to negotiations being entered as to price. Both companies selected technology utilizing a claimed method based upon their evaluations of performance. Thus, so far as is understood, price was not a factor for selection of a presently claimed method over the UBI technology.

It is thus seen that there is and was more than enough evidence to illustrate commercial success for the claimed subject matter.

D. Further Evidence of Unobviousness

Dr. Helting's enclosed Second Declaration also provides still further evidence of the unobviousness of the claimed subject matter, particularly the recombinant 1-120 protein. This Declaration and its underlying work were undertaken in response to points raised in the Final Action, and illustrate, inter alia, the differences in environmental effects upon the binding of HCV

antibodies to peptide or recombinant antigens, and further illustrates the superiority of the latter.

Thus, equivalent amounts of the recombinant 1-120 protein (Preparation A), a 21-40 recombinant fusion protein (Preparation C) and the free 21-40 peptide prepared by cleavage of the recombinant fusion protein (Preparation B) were prepared. Four equivalent concentrations of each preparation (12 in all) were spotted vertically on nitrocellulose strips as antigens and the strips were separately immunoreacted with each of four sera known to immunoreact with the capsid.

The results of that study showed the 1-120 recombinant and the recombinant fusion protein to exhibit excellent binding with 3 of 4 sera, and almost no immunoreactivity with the free peptide with any serum. Dr. Helting concluded from those results that

(i) that the pattern of reactivity of an amino acid residue sequence seen within the context of one molecular framework of a recombinant protein or fusion protein cannot be used to predict what reactivity pattern might emerge if the same sequence is assayed in a molecular environment that lacks the fused recombinant protein portion;

(ii) the free residues 21-40 peptide of Preparation B is similar to entities derived by chemical synthesis, and in this study such a peptide is clearly inferior to maintain the immune reactivity it possesses within the context of the larger protein structure of the recombinant whole protein or fusion protein;

(iii) in view of the previously shown similarities between the 1-120 recombinant and the recombinant 1-74 fusion protein, the immunodominant domain for the HCV capsid lies in the region between position 1 and position 74 of the protein;

(iv) in view of the similar reactivities shown here between the 1-120 recombinant and the recombinant 21-40 fusion protein, that immunodominant region is most likely centered at about positions 21-40 of the capsid;

(v) it is his belief in view of the data in the subject application, its co-pending continuation-in-part Serial No. 07/616,369, and the submitted articles and data, including those data herein, that the longer chains of amino acid residues present in the recombinant 1-74 fusion protein, the recombinant 21-40 fusion protein and recombinant 1-120 protein on one side or the other of the immunodominant domain function to optimally present the immunodominant domain to the antibodies; and

(vi) that such optimal presentation is not achieved with the smaller polypeptides, such as the synthetic polypeptides disclosed in the Wang patent.

Dr. Helting's studies further illustrate that having the immunodominant region present as a peptide antigen, as disclosed by Wang, is a necessary but insufficient condition for obtaining a useful assay. Rather, epitope of that peptide antigen must be properly presented to the antibodies for the assay to work appropriately. The epitope of the free peptide is not so presented, whereas the added length provided by the fused protein or C-terminal sequence of the whole protein does so present the epitope.

Had one followed the conclusions proposed in the various Actions here, the 21-40 peptide should have reacted similarly to its fusion protein or the 1-120 recombinant. It did not.

Thus, again, the Wang results must be narrowly construed, limited to what is actually shown and cannot be used predictively for other systems. When that is done, it is seen

that the presently claimed subject matter provides unexpectedly different, superior and unobvious results, and the claimed subject matter should be patentable.

III. SUMMARY

Claim 28 has been amended and claim 29 cancelled. The various points raised in the Action have been dealt with and the rejections overcome. Further evidence of the unpredictability of the antigen-antibody interactions at play here have been provided, as has further evidence of commercial success.

It is therefore submitted that the subject application is in condition for allowance. An early notice to that effect is earnestly solicited.

The Examiner is urged to phone the undersigned should she have any questions or suggestions for further amendments that may speed allowance.

No further fee or petition is believed necessary. However, should any further fee be needed, please charge our Deposit Account No. 04-1644, and deem this paper the required Petition.

Respectfully submitted,

By Edward P. Gamson
Edward P. Gamson, Reg. No. 29,381

Enclosures


Declaration of Dr. Torsten B. Helting
Fee Under 37 C.F.R. §1.97(c)

CERTIFICATE OF MAILING

I hereby certify that this Amendment Under 37 C.F.R. §1.116, together with the stated enclosures, is being deposited with the United States Postal Service as First Class Mail, postage prepaid, in an envelope addressed to: Box AF, Hon. Commissioner of Patents and Trademarks, Washington, D.C. 20231 on October 12, 1993.

Edward P. [Signature]

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee )
Serial No.: 07/573,6) Attorney Docket
) PHA 0025P
Filed: August 27, 1990) Group Art Unit: 1802
)
For: NON-A, NON-B, HEPATITIS VIRUS)
ANTIGEN, DIAGNOSTIC METHODS AND)
VACCINES)
Examiner: D. Wortman)

SECOND DECLARATION OF DR. TORSTEN B. HELTING

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

DR. TORSTEN B. HELTING, Declares:

1. That he is the Dr. Torsten B. Helting whose prior Declaration is already of record herein;
2. That he has read and is familiar with the Official Action dated July 13, 1993;
3. That because of the points raised in that Action, he carried out the further studies discussed below;
4. That free capsid protein residues 1-120 were isolated from induced E. coli cultures transformed with a plasmid encoding the amino acid sequence from position 1 through position 120 of Figure 1 of the application, and that a solution containing the free recombinant capsid protein was subsequently isolated via gel and ion exchange chromatography;

#26
2/11/93

BOX AP

10/2/93
C. J. [signature]

5. That the purified capsid was adjusted to an $OD_{280\text{ nm}}$ equal to 0.01 by diluting with 20 mM Tris-HCl buffer, pH 7.5 containing 500 mM NaCl (TBS), and labeled Preparation A;

6. That the GST-21-40 fusion protein of copending application Serial No. 07/616,369 was isolated from induced E. coli cultures transformed with the plasmid GST-2T-CAP-B of that application; the bacteria being harvested by centrifugation and being subsequently treated with lysozyme and ultrasonication, differential centrifugation, Sepharose S-300 gel chromatography and affinity chromatography on a glutathione-agarose affinity column (Cat. #9761, Sigma). The latter column was washed with 0.02M Tris-HCl buffer, containing 0.2 M NaCl and the purified GST-21-40 fusion protein was eluted from the affinity column by using the same buffer containing reduced glutathione (5 mg/ml) (enclosed Fig. 1). The eluate (Peak 2, Fig. 1, attached) was subsequently dialyzed against 0.05 M Tris-HCl, pH 7.2 containing 0.15 M NaCl and 2.5 mM $CaCl_2$;

7. That five ml of the dialyzed preparation, having an OD_{280} equal to 0.06, were retained as a parent GST-21-40 preparation during the incubation of an equal volume (5 ml) with human thrombin (100 U/ml, 25 μ l) at room temperature for 60 minutes. The resulting thrombin digest was then applied to a column (0.8x5 cm) of the glutathione-agarose affinity resin. The flow-through was collected and the column washed to elute the released peptide in a total volume of 10 ml (i.e. a 1:2 dilution of the starting material (enclosed Fig. 2, peak 1; prep I). The

free peptide was thus separated from the GST carrier, which still bound to the affinity resin and was eluted with buffer containing reduced glutathione (5 mg/ml) ^{on} Fig. 2, peak 2);

8. That the parent GST-21-40 preparation was also diluted 1:2 (5 ml adjusted to 10 ml), thus providing an equivalent molar concentration of free peptide and fusion protein (prep. II);

9. That subsequently, to adjust all preparations to an approximate OD₂₈₀ equal to 0.01 (or equivalent), a 1:3 dilution of prep. I in TBS was prepared and labeled Preparation B;

10. That likewise, a 1:3 dilution of prep. II, described above was prepared by addition of TBS and labeled Preparation C. Thus, Preparations B and C constitute equivalent molar concentrations of the 21-40 peptide, present in Preparation B in free form, whereas linked to GST in Preparation C;

11. That the recombinant capsid preparations were subsequently compared as antigens by adsorption onto nitrocellulose membrane at four different concentrations and subsequently incubated with four different capsid reactive HCV sera to investigate the relative immune reactivity as follows:

(a) A sheet of nitrocellulose membrane (Sigma Cat. #N8017) was wetted with TBS and mounted in a Biorad-Biodot microfiltration apparatus (Cat. #170545). In each row of 12 wells, the antigen-containing samples were applied as follows:

Well	Preparation	Dilution in TBS	Volume per Well
1	A	Neat	0.1
2	A	1:5	0.1
3	A	1:25	0.1
4	A	1:125	0.1
5	B	Neat	0.1
6	B	1:5	0.1
7	B	1:25	0.1
8	B	1:125	0.1
9	C	Neat	0.1
10	C	1:5	0.1
11	C	1:25	0.1
12	C	1:125	0.1

(b) After application of the samples and blocking with 1 percent bovine serum albumin (BSA) in TBS buffer, the wells were washed with TBS containing 0.1 percent Tween 20 (TTBS), and the nitrocellulose sheet removed and dried over P_2O_5 overnight.

(c) Strips containing the 12 samples were cut and subjected to an immunoreaction as follows:

(i) Using a Biorad mini-incubation tray, (Cat # 170-3902), to each of four troughs were added 1 ml of TTBS containing 1 percent BSA and 10 μ l of a random member of a serum reference panel known to react with the structural region of HCV (Ortho Riba II test). Each trough received one strip, each strip containing the three antigens applied

in four different concentrations as shown in the table above;

(ii) After incubating for three hours at 30°C on an orbital shaker, the liquid in each trough was aspirated and the strips washed 5x with phosphate buffered saline containing 0.1 percent Tween 20. Subsequently, blotting grade anti-human IgG-alkaline phosphatase conjugate (Biorad,

Or Cat. #170-6521) diluted 1:1000 in fresh TBBS containing 1 per cent BSA (1.5 ml) was added and the incubation continued for an additional 60 minutes.

After removal of the enzyme conjugate and five washes with PBS Tween, the strips were developed by adding the BCIP/NBT (Biorad Cat. #170-6539, 170-6532, respectively) substrate in accordance with the manufacturer's instructions, and incubating for 20 minutes. The reaction was terminated by washing the strips with water and drying;

12. That Fig. 3 shows the original mounted strips obtained with those four random sera known to react with the HCV structural region;

13. That although the immune reactivity shows the expected variation depending on the serum used, the results show a consistent pattern of reactivity, in three out of four sera

down to a dilution of 1:125 when using the GST-21-40 fusion protein or a claimed recombinant 1-120 protein for application to the membrane;

14. That by comparison, the isolated free peptide (residues 21-40, Preparation B) derived from Preparation C shows an almost negligible level of activity under identical conditions;

15. That it is concluded:

(i) that the pattern of reactivity of an amino acid residue sequence seen within the context of one molecular framework of a recombinant protein or fusion protein cannot be used to predict what reactivity pattern might emerge if the same sequence is assayed in a molecular environment that lacks the fused recombinant protein portion;

(ii) the free residues 21-40 peptide of Preparation B is similar to entities derived by chemical synthesis, and in this study such a peptide is clearly inferior to maintain the immune reactivity it possesses within the context of the larger protein structure of the recombinant whole protein or fusion protein;

(iii) in view of the previously shown similarities between the 1-120 recombinant and the recombinant 1-74 fusion protein, the immunodominant domain for the HCV capsid lies in the region between position 1 and position 74 of the protein;

(iv) in view of the similar reactivities shown here between the 1-120 recombinant and the recombinant 21-40

fusion protein, that immunodominant region is most likely centered at about positions 21-40 of the capsid;

(v) it is his belief in view of the data in the subject application, its co-pending continuation-in-part Serial No. 07/616,369, and the submitted articles and data, including those data herein, that the longer chains of amino acid residues present in the recombinant 1-74 fusion protein, the recombinant 21-40 fusion protein and recombinant 1-120 protein on one side or the other of the immunodominant domain function to optimally present the immunodominant domain to the antibodies; and

(vi) that such optimal presentation is not achieved with the smaller polypeptides, such as the synthetic polypeptides disclosed in the Wang patent;

16. That he is aware of no evidence, including the Wang patent, that suggests the presence of one or more discontinuous linear or conformational epitopes being present in the region of the HCV capsid protein from position 74 to the C-terminus at position 120 that can be of direct significance to an immunoassay;

17. That he is aware of no evidence of any epitope being present in the region of the HCV capsid protein from position 74 to the C-terminus at position 120 that:

(i) approaches the reactivity with antibody exhibited by the recombinant GST-1-74 or GST-21-40 fusion proteins or the recombinant 1-120 protein,

(ii) that may exhibit strong antibody binding properties, and

(iii) be of direct significance to an immunoassay, that lack of evidence being bolstered by the negative binding results obtained using the CAP-B (GST-69-120) fusion protein discussed in document BC, and particularly Table 1 of that document;

18. That he views the results illustrated in Wang's Table 7 that gave rise to her assertions as to the possible presence of discontinuous linear or conformational epitopes evidenced generally poor antibody binding so that epitopes of minor importance to an immunoassay were observed;

19. That upon information and belief gained from those with whom he has had license or option negotiations, it is his understanding:

(i) that data on the UBI technology were assembled and evaluated by Sorin Biomedica and Behringwerke, the present licensee and optionee of technology utilizing a method of the present claims,

(ii) that the UBI technology and that of a method of the present claims were assessed for performance prior to entering negotiations as to price, and

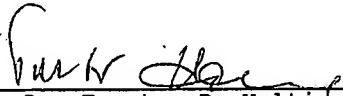
(iii) that technology utilizing a claimed method was selected over the UBI technology based upon their respective performances;

Serial No. 07/573,643

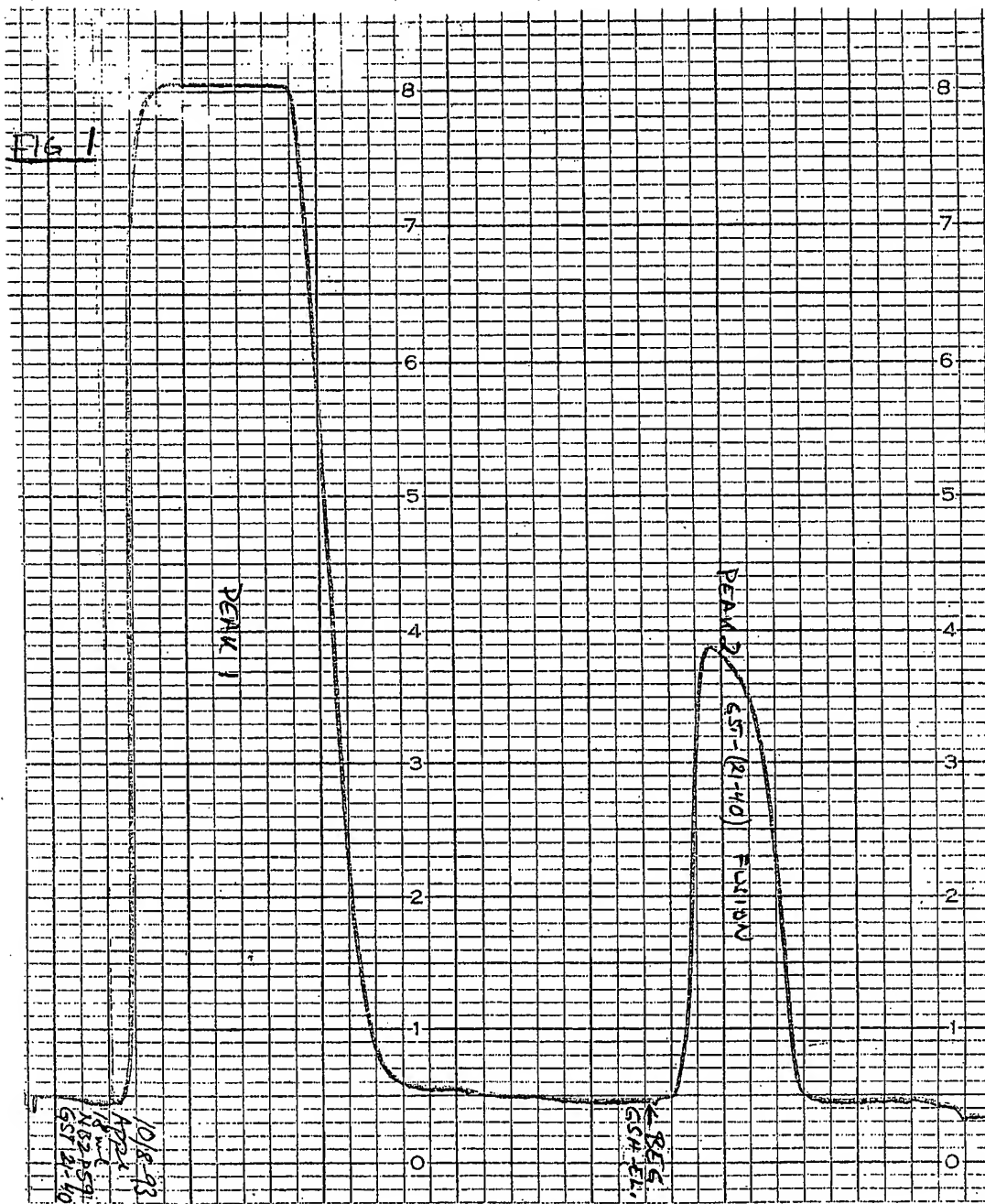
-9-

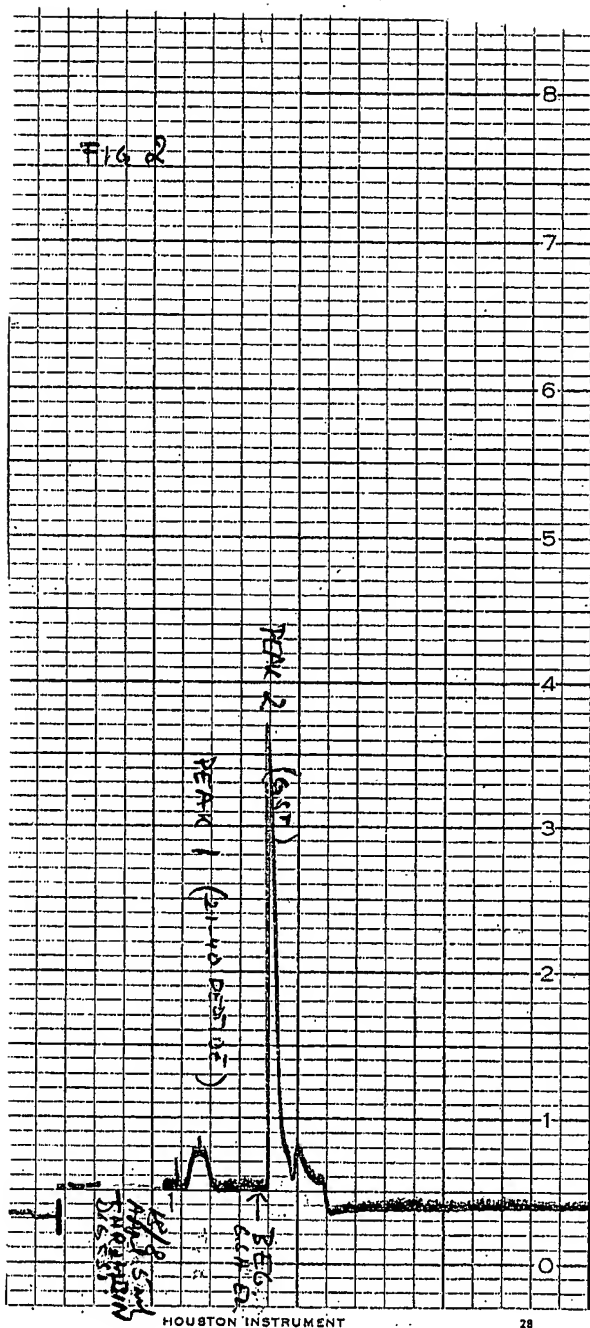
20. That he further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing on this application.

October 8, 1993
Date


Dr. Torsten B. Helting

Enclosures



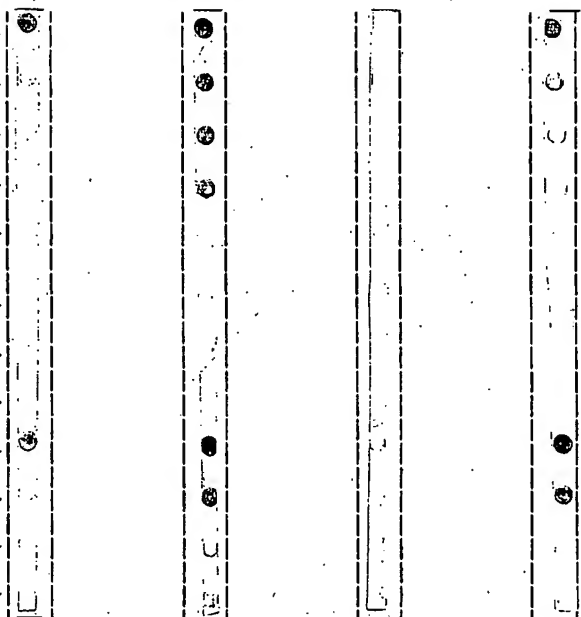


Dot Blot Analysis

Date: Aug 18, 1993
 Ref. NR 2 PL
DOT BLOT PREP A-C

Antigen:

A, Neat _____
 2 A 1:5 _____
 3 A 1:25 _____
 4 A 1:125 _____
 5 B Neat _____
 6 B 1:5 _____
 7 B 1:25 _____
 8 B 1:125 _____
 9 C Neat _____
 10 C 1:5 _____
 11 C 1:25 _____
 12 C 1:125 _____



Serum ID:

M34727

M34890

9748

MRB3F3

5-3693



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
07/573,643	08/27/90	ZEBEDEE	S PHA0025P

18N1/1101
DRESSLER, GOLDSMITH, SHORE,
SUTKER & MILNAMOW, LTD.
11300 SORRENTO VALLEY RD., STE. 200
SAN DIEGO, CA 92121

WORD NUMBER	
ART UNIT	PAPER NUMBER
1802	25

DATE MAILED:

11/01/93

Below is a communication from the EXAMINER in charge of this application

COMMISSIONER OF PATENTS AND TRADEMARKS

ADVISORY ACTION

☒ THE PERIOD FOR RESPONSE:

- a) ☐ is extended to run _____ or continues to run _____ from the date of the final rejection
- b) ☒ expires three months from the date of the final rejection or as of the mailing date of this Advisory Action, whichever is later. In no event however, will the statutory period for the response expire later than six months from the date of the final rejection.

Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a), the proposed response and the appropriate fee. The date on which the response, the petition, and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee. Any extension fee pursuant to 37 CFR 1.17 will be calculated from the date of the originally set shortened statutory period for response or as set forth in b) above.

☐ Appellant's Brief is due in accordance with 37 CFR 1.192(a).

☒ Applicant's response to the final rejection, filed 10-12-93 has been considered with the following effect, but it is not deemed to place the application in condition for allowance:

1. ☐ The proposed amendments to the claim and/or specification will not be entered and the final rejection stands because:
- a. ☐ There is no convincing showing under 37 CFR 1.116(b) why the proposed amendment is necessary and was not earlier presented.
 - b. ☐ They raise new issues that would require further consideration and/or search. (See Note).
 - c. ☐ They raise the issue of new matter. (See Note).
 - d. ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.
 - e. ☐ They present additional claims without cancelling a corresponding number of finally rejected claims.

NOTE:

2. ☐ Newly proposed or amended claims _____ would be allowed if submitted in a separately filed amendment cancelling the non-allowable claims.

3. ☒ Upon the filing an appeal, the proposed amendment ☒ will be entered ☐ will not be entered and the status of the claims will be as follows:

Claims allowed: _____
Claims objected to: _____
Claims rejected: 28, 30-37

However,

☒ Applicant's response has overcome the following rejection(s): rejection under 35 USC 112

4. ☒ The affidavit, exhibit or request for reconsideration has been considered but does not overcome the rejection because See attached

5. ☐ The affidavit or exhibit will not be considered because applicant has not shown good and sufficient reasons why it was not earlier presented.

☐ The proposed drawing correction ☐ has ☐ has not been approved by the examiner.

☒ Other PTO 1449x2, copies attached.
Sheet

The Information Disclosure Statements previously submitted as Paper No. 18 and attached to Paper No. 20 have now been made of record and copies are attached to this Advisory Action.

5 The proposed amendment cancelling Claim 29 and amending Claim 28 would overcome rejection of Claim 28 and claims dependent thereon as previously made under 35 USC 112.

10 The proposed amendment clarifying and narrowing the scope of Claim 28 to recite alternatively recombinant protein 1-120 or fusion protein gst-1-74, Applicant's remarks, and the Second Declaration of Dr. Helting have been considered but have not been found persuasive of unobviousness over Wang in view of Kuo et al.

15 Dr. Helting's declaration presents data comparing the binding of equivalent molar concentrations recombinant capsid protein 1-120, Preparation A; recombinant free peptide 21-40, Preparation B; and recombinant fusion protein GST-21-40, preparation C to four different capsid reactive HCV sera. The results show that recombinant capsid protein 1-120 is most
20 reactive, that recombinant fusion protein GST-21-40 is almost as reactive as 1-120, and that GST-21-40 is considerably better than free 21-40. However, the declaration does not provide a direct comparison of the closest prior art with the recombinant fusion protein having the amino acid sequence represented in the
25 sequence in Figure 1 from 1-120 or the fusion protein sequence shown in Figure 2 from 1-315 as Applicant now proposes to claim. In particular, as referenced in the Office actions Papers No. 14 and 22, Wang teaches assaying sera for antibodies against HCV (NANBV) using solid phase coated with synthetic peptides that are very close to the amino acid residue sequences as instantly
30 claimed (see Wang, Example 14 and Table 7, especially peptides VIIIE, IXD, and IXE) and mixtures of peptides. One would expect recombinant protein 1-120 to give results at least as good as a mixture of two synthetic peptides, one encompassing approximately
35 amino acids 1-60 and the other representing approximately 60-120, and one might reasonably expect recombinant protein 1-120 to give results somewhat better than a mixture of two such synthetic proteins since one might expect at least one epitope to be
40 interrupted by the discontinuity in the middle of the sequence. Separately considering fusion protein GST-1-74, one would expect it to produce results at least as good as those given by peptide VIIIE and probably somewhat better since it contains more amino acids and presumably one or more additional epitopes. There is
45 no convincing evidence of record showing such unexpected results.

The arguments that Wang, in listing the advantages of using synthetic antigens, teaches away from using recombinant antigens are not found persuasive because, while it is true that certain
50 advantages are associated with using synthetic antigens, one of

Serial No. 573643
Art Unit 1802

-3-

ordinary skill in the art at the time the invention was made would have recognized that there are advantages as well to using recombinant antigens as previously discussed, and if one were willing to forego the advantages of synthetics in favor of the known advantages of recombinants, then one would have been adequately motivated to select recombinant antigens. Wang herself realized that recombinant antigens may be substituted for synthetic antigens: Wang, col. 25, lines 29-42.

In addition, arguments referring to Wang's results as compared to C100 and the instant antigen's results compared to C100 are not fully persuasive because it appears that the assays of Wang and the assays done using the instant antigens were not done using the same sera (See Wang, col. 18, lines 9-15, with reference to the results presented in Table 7: "Each of these peptides ... with a panel of HCV antibody positive sera, each selected as representative of a particular clinical population, ..."; the source of the sera used by Dr. Helting is not immediately clear.). The support for Applicant's remarks on page 10 asserting that recombinant 1-120 "also outperformed Wang's two peptide mixture (VIII E and IX D) as to Chimp 10" is not immediately apparent; Applicant is requested to point out the location of results which show that particular comparison to assist the Examiner in evaluating the evidence regarding recombinant 1-120 more completely.

In re Bell as cited by Applicant is not believed to apply here since the entire nucleotide as well as the entire amino acid sequence of HCV was generally known at the time the invention was made (see Wang, paragraph bridging col. 3-4).

Applicant's assertion of commercial success as further indication of unobviousness cannot be completely evaluated because the evidence has not been made available. Dr. Helting's Second Declaration has been considered in that regard but is not found to be sufficient evidence since it is not known what considerations were taken into account in the licensee and optionee decision nor is it known exactly what performance comparisons were made. In addition, Dr. Helting's Declaration is not commensurate in scope with the claims since it only concerns "a" claimed method when the claims recite two distinctly different recombinant antigens.

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform to the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 308-4227.

Serial No. 573643
Art Unit 1802

-4-

Any inquiry concerning this communication should be directed
to Examiner Donna C. Wortman at telephone number (703) 308-1032.

5

10 Donna C. Wortman, Ph.D.
October 27, 1993



ESTHER L. KEPPLINGER
SUPERVISORY PATENT EXAMINER
GROUP ART UNIT 1802

840-117-

1802 #26
29-94



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

FEB 01 1994

Applicant: Zebedee et al.)
Serial No.: 07/573,643) Attorney Docket
Filed: August 27, 1990) PHA 0025P
For: NON-A, NON-B, HEPTATITIS VIRUS)
ANTIGEN, DIAGNOSTIC METHODS AND)
VACCINES)
Examiner: D. Wortman)

PETITION UNDER 37 C.F.R. §1.17

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

A three-month extension of time to respond to the Final Rejection mailed July 13, 1993 is respectfully requested. This Petition is requested in order to allow the applicants to file a Notice of Appeal, enclosed herewith.

There is submitted herewith the following:

1. Check No. 21519 in the amount of the required fee of \$840.00 for the three-month extension of time (a response to the Final Rejection was due on October 13, 1993);
2. Notice of Appeal, in duplicate; and
3. Check No. 21518 in the amount of \$270.00 for the fee for the Notice of Appeal.

The Commissioner is hereby authorized to charge payment of any additional fees or credit any overpayment to Deposit Account No. 23-0920. A duplicate copy of this paper is enclosed.

Serial No. 07/573,643

-2-

Please note that the undersigned has moved his practice to the address below, while maintaining the Power of Attorney in this application. Please forward all further communications concerning this application to counsel at the address shown below.

Respectfully submitted,

By Edward P. Gamson
Edward P. Gamson, Reg. No. 29,381

WELSH & KATZ, LTD.
Suite 1625
135 South La Salle Street
Chicago, Illinois 60603
312/781-9470

CERTIFICATE OF MAILING

I hereby certify that this Petition, in duplicate, together with the aforementioned documents and the required fees, is being deposited with the United States Postal Service as First Class Mail, postage prepaid, on January 11, 1994, addressed to Hon. Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Edward P. Gamson

270-119

PHA-0025P
14829297

1800 #21
2-9-94



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

FEB 1 1994

GROUP 1800

Applicant: Zebedee et al.

07/573,643

Filed: August 27, 1990

For: NON-A, NON-B, HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

Group Art Unit: 1802

Examiner: D. Wortman

Attorney
Docket No.: PHA-0025P

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on this date.

1/11/94 Edward D. Johnson
Date Registration No. 29381
Attorney for Applicant(s)

NOTICE OF APPEAL FROM THE PRIMARY EXAMINER TO THE BOARD OF APPEALS

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

7/13/93 Applicant hereby appeals to the Board of Appeals from the decision dated of the Primary Examiner finally rejecting claims 28 and 30-37, inclusive

The item(s) checked below are appropriate:

1. (X) A Petition for Extension of Time to respond to the final rejection is filed herewith.
2. (X) Fee amount \$270.00
(X) Enclosed.
() Not required (fee paid in prior appeal).
(X) The Commissioner is hereby authorized to charge any additional fee which may be required, or credit any overpayment to Deposit Account No. 23-0920. Should no proper payment be enclosed, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 23-0920. (One additional copy of this Notice is enclosed herewith.)

Dated: January 11, 1994

Edward D. Johnson
Registration No. 29,381

Address to which Correspondence is to be sent:

WELSH & KATZ, LTD.
135 South LaSalle Street
Suite 1625
Chicago, Illinois 60603
(312) 781-9470

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7/28

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Appl To 171



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee et al.)
Serial NO. 07/573,643) Attorney Docket
Filed: August 27, 1990) PHA 0025P
(2673/59297)
For: NON-A, NON-B, HEPATITIS VIRUS) Group Art Unit 1802
ANTIGEN, DIAGNOSTIC METHODS AND)
VACCINES)
Examiner: D. Wortman)

PETITION UNDER 37 C.F.R. §1.17

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

RECEIVED
JUL 27 1994
COMM. OF PAT. & TRADEMARKS

Sir:

A four-month extension of time to file the Appellants' Brief on Appeal is respectfully requested. The Brief was due March 11, 1994. This extension of time is requested to allow the appellants to file a continuation application under 37 C.F.R. 1.62 enclosed herewith.

Enclosed is Check No. 23760 in the amount of the required fee of \$1,320.00 for the four-month extension of time.

The Commissioner is hereby authorized to charge payment of any additional fees under 37 C.F.R. §1.17 to cover the cost of the extension or credit any overpayment to Deposit Account No. 23-0920. A duplicate copy of this paper is enclosed.

Respectfully submitted,

By

Edward P. Gamson, Reg. No. 29,381

WELSH & KATZ, LTD.
135 South La Salle Street
Chicago, Illinois 60603
312/781-9470

Serial No. 07/573,643

-2-

CERTIFICATE OF MAILING BY EXPRESS MAIL

I hereby certify that this Petition, in duplicate, together with the aforementioned documents and the required fees, is being deposited as Express Mail No. TB59847545X on July 8, 1994, addressed to Box FWC, Hon. Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Robert J. Smith

FORM PTO-875 (REV. 11-85)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO 573643	FILED DATE 8-27-90
PATENT APPLICATION FEE DETERMINATION RECORD		APPLICANT (FIRST NAMED) S. Z. Shedee et al	

CLAIMS AS FILED - PART I

FOR	NO FILED	NO EXTRA
BASIC FEE		
TOTAL CLAIMS	74	34
INDEP. CLAIMS	7	4
MULTIPLE DEPENDENT CLAIMS PRESENT		

* If the difference in col. 1 is less than 20, enter "0" in col. 2

SMALL ENTITY

RATE	FEE
	\$ 185
x 6	\$
x 18	\$
60	\$
TOTAL	\$

OTHER THAN A SMALL ENTITY

RATE	FEE
	\$ 370
x 12	\$ 648
x 36	\$ 1,440
120	\$ 20
TOTAL	\$ 1,752

CLAIMS AS AMENDED - PART II

AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NO PREVIOUSLY PAID FOR	PRESENT EXTRA
TOTAL	8	74	
INDEP	1		
FIRST PRESENTATION OF MULTIPLE DEP. CLAIM			

SMALL ENTITY

RATE	ADDIT. FEE
x 6	\$
x 18	\$
60	\$
TOTAL	\$

OTHER THAN A SMALL ENTITY

RATE	ADDIT. FEE
x 12	\$
x 36	\$
120	\$
TOTAL	\$

AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NO PREVIOUSLY PAID FOR	PRESENT EXTRA
TOTAL			
INDEP			
FIRST PRESENTATION OF MULTIPLE DEP. CLAIM			

RATE	ADDIT. FEE
x 6	\$
x 18	\$
60	\$
TOTAL	\$

RATE	ADDIT. FEE
x 12	\$
x 36	\$
120	\$
TOTAL	\$

AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NO PREVIOUSLY PAID FOR	PRESENT EXTRA
TOTAL			
INDEP			
FIRST PRESENTATION OF MULTIPLE DEP. CLAIM			

RATE	ADDIT. FEE
x 6	\$
x 18	\$
60	\$
TOTAL	\$

RATE	ADDIT. FEE
x 12	\$
x 18	\$
60	\$
TOTAL	\$

* If the entry in Col. 1 is less than the entry in Col. 2, enter "0" in Col. 3

** If the highest fee previously paid for 15 THIS SPACE is less than 20, enter "20"

*** If the highest fee previously paid for 15 THIS SPACE is less than 3, enter "3"

PTO-1130 (REV. 8-87)	PALM III APPLICATION FILE DATA CODING SHEET		U.S. DEPARTMENT OF COMMERCE-PATENT & TM OFFICE	FILED BY <i>W. H. H. / Ivey</i>	DATE <i>6-17-90</i>
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PTO-1130 (REV. 8-87)	PALM III APPLICATION FILE DATA CODING SHEET		U.S. DEPARTMENT OF COMMERCE-PATENT & TM OFFICE	FILED BY <i>W. H. H. / Ivey</i>	DATE <i>6-17-90</i>
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PTO-1130 (REV. 8-87)	PALM III APPLICATION FILE DATA CODING SHEET		U.S. DEPARTMENT OF COMMERCE-PATENT & TM OFFICE	FILED BY <i>W. H. H. / Ivey</i>	DATE <i>6-17-90</i>
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PTO-1130 (REV. 8-87)	PALM III APPLICATION FILE DATA CODING SHEET		U.S. DEPARTMENT OF COMMERCE-PATENT & TM OFFICE	FILED BY <i>W. H. H. / Ivey</i>	DATE <i>6-17-90</i>
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PTO-1130 (REV. 8-87)	PALM III APPLICATION FILE DATA CODING SHEET		U.S. DEPARTMENT OF COMMERCE-PATENT & TM OFFICE	FILED BY <i>W. H. H. / Ivey</i>	DATE <i>6-17-90</i>
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PATENT APPLICATION SERIAL NO. 08/272275

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

010 TL 07/20/94 08272275

1 101 710.00 CK

PTO-1556
(5/87)



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

July 8, 1994

Date of Deposit

"Express Mail" mailing number

I hereby certify that this application is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Box FWC, Washington, D.C. 20231.

Robert Smith

Typed or printed name of person mailing application

Robert Smith

Signature of person mailing application

Anticipated Classification of this Application:

Class _____ Subclass _____

Prior Application:

Examiner D. Wortman

Art Unit 1802

COMMISSIONER OF PATENTS AND TRADEMARKS

Box FWC

Washington, D.C. 20231

Sir:

**FILING UNDER 37 CFR 1.62 WITH
ABANDONMENT OF THE PENDING PRIOR APPLICATION**

This is a Request for filing a ☐ continuation-in-part, ☒ continuation, ☐ divisional application under 37 CFR 1.62 of prior application Serial No. 07/573,643, filed on August 27, 1990 entitled NON-A, NON-B, HEPATITIS VIRUS ANTIGEN; DIAGNOSTIC METHODS AND VACCINES by the following named inventor(s).

FULL NAME OF INVENTOR	FAMILY NAME <u>Zebedee</u> ✓	FIRST GIVEN NAME <u>Suzanne</u>	SECOND GIVEN NAME --
RESIDENCE & CITIZENSHIP	CITY <u>San Diego</u>	STATE OR FOREIGN COUNTRY <u>California CA</u>	COUNTRY OF CITIZENSHIP <u>U.S.A.</u>
POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>7544 Charmant Drive</u>	CITY <u>San Diego</u>	STATE & ZIP CODE/COUNTRY <u>California 92122</u>
FULL NAME OF INVENTOR	FAMILY NAME <u>Inchausepe</u> ✓	FIRST GIVEN NAME <u>Genevieve</u>	SECOND GIVEN NAME --
RESIDENCE & CITIZENSHIP	CITY <u>New York</u>	STATE OR FOREIGN COUNTRY <u>New York NY</u>	COUNTRY OF CITIZENSHIP <u>France</u>
POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>504 East 63rd Street</u>	CITY <u>New York</u>	STATE & ZIP CODE/COUNTRY <u>New York 10021</u>
FULL NAME OF INVENTOR	FAMILY NAME <u>Nasoff</u> ✓	FIRST GIVEN NAME <u>Marc</u>	SECOND GIVEN NAME <u>S.</u>
RESIDENCE & CITIZENSHIP	CITY <u>San Diego</u>	STATE OR FOREIGN COUNTRY <u>California CA</u>	COUNTRY OF CITIZENSHIP <u>U.S.A.</u>
POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>11734 Mira Lago Way</u>	CITY <u>San Diego</u>	STATE & ZIP CODE/COUNTRY <u>California 92131</u>

continued ...

Page 1 of 3

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08/272275

#296

Off.

\$2596

sent EOT To MP. 1803

continued from page 1

4-00

FULL NAME OF INVENTOR	FAMILY NAME <u>Prince</u> ✓	FIRST GIVEN NAME <u>Alfred</u>	SECOND GIVEN NAME M.
RESIDENCE & CITIZENSHIP	CITY New York	STATE OR FOREIGN COUNTRY NY New York	COUNTRY OF CITIZENSHIP U.S.A.
POST OFFICE ADDRESS	POST OFFICE ADDRESS Pound Ridge 154 Stone Gill Road /	CITY New York	STATE & ZIP CODE/COUNTRY New York 10576
FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY

The above-identified prior application in which no payment in the issue fee, abandonment of, or termination of proceedings has occurred, is hereby expressly abandoned as of the filing date of this new application. Please use all the contents of the prior application file wrapper, including the drawings, as the basic papers for the new application. (Note: 37 CFR 1.60 may be used for applications where the prior application is not to be abandoned.)

1. ☒ Enter the amendment previously filed on October 12, 1993 under 37 CFR 1.116 but unentered, in the prior application.
2. ☐ A preliminary amendment is enclosed.

The filing fee is calculated on the basis of the claims existing in the prior application as amended at 1 and 2, above.

CLAIMS	Claim Type	Number Filed	Number Extra	Rate	Calculations
	Total Claims	9 - 20 =	-0-	X \$ 22.00	\$ --
	Independent Claims	1 - 3 =	-0-	X \$ 74.00	--
	Multiple Dependent Claim(s) (if applicable)			+ \$230.00	--
				Basic Fee	+ 710.00
				Total of Above Calculations =	\$710.00
	Reduction by 1/2 for filing by small entity (Note 37 CFR 1.9, 1.27, 1.28); if applicable, affidavit must be filed also.				- --
				Total National Fee	\$ 710.00

3. ☒ The Commissioner is hereby authorized to charge fees under 37 CFR 1.16 and 1.17 which may be required, or credit any overpayment to Deposit Account No. 23-0920.
4. ☒ A check in the amount of \$ 710.00 is enclosed.
5. ☐ A new oath or declaration is included since this application is a continuation-in-part which discloses and claims additional matter.
6. ☒ Amend the specification by inserting before the first line the sentence:

-- This application is a ☐ continuation-in-part, ☒ continuation, ☐ division, of application
Serial No. 07/573,643, filed August 27, 1990

7. ☐ A verified statement claiming small entity status is enclosed (not necessary if statement was filed in the prior application).
8. ☐ Priority of application Serial No. _____ filed on _____ in _____ is claimed under 35 U.S.C. 119.
9. ☒ The prior application is assigned of record to The New York Blood Center
10. ☒ The power of attorney in the prior application is to: Edward P. Gamson, Reg. No. 29,381

11. ☐ The small entity statement was filed in the parent application Serial No. _____ on _____
_____ and this status is still proper and its benefit under 37 CFR 1.28(a) is hereby claimed.

12. ☒ Also enclosed:
Petition, in duplicate, for a four-month extension of time in
Serial No. 07/573,643, and fee

Address all future communications to: (May only be completed by applicant, or attorney or agent of record.)

WELSH & KATZ, LTD.
Suite 1625
135 South La Salle Street
Chicago, Illinois 60603

Telephone: 312/781-9470

It is understood that secrecy under 35 U.S.C. 122 is hereby waived to the extent that if information or access is available to any one of the applications in the file wrapper of a 37 CFR 1.62 application, be it either this application or a prior application in the same file wrapper, the Patent and Trademark Office may provide similar information or access to all the other applications in the same file wrapper.

Date: July 8, 1994



Attorney's Signature

Name and Reg. No. Edward P. Gamson, 29,381



CP 188

30
7/18/94
10/24/94

7/18/94
10/24/94

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee et al.)
Serial No.: 08/272,275) Attorney Docket
) PHA-0025P CON I
Filed: July 8, 1994) Group Art Unit:
) Not yet assigned
For: NON-A, NON-B, HEPATITIS VIRUS)
ANTIGEN, DIAGNOSTIC METHODS AND)
VACCINES)
Examiner: Not yet assigned)

INFORMATION DISCLOSURE STATEMENT

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

7/18/94
OCT 11 1994
APPL. DIV.

Sir:

Pursuant to 37 C.F.R. §1.97, a list of documents is disclosed on the attached forms PTO-1449 that may be material to the examination of this application. The subject application is a continuation application of Serial No. 07/573,643, filed August 27, 1990 that was one of three related applications referred to herein as the "grandparent", "parent", and "child" applications. The serial numbers and filing dates of those applications are 07/573,643, filed on August 27, 1990 (the grandparent application), 07/616,369 filed on November 21, 1990 (the parent application and a C-I-P of the grandparent application), and 07/748,564 filed on August 21, 1991 (the child application and a C-I-P application of the parent application).

Listed documents A and D-N on the attached form PTO-1449 are cited and discussed in all three applications.

Listed documents O-Z are recited and discussed only the child application.

Listed documents AA-AG are included on the list as general background art related to the work of some of the present inventors on non-A/non-B hepatitis viruses.

Listed documents B, C and AH were cited in the International Search Report for PCT Application PCT/US91/06037, which application corresponds to the child application. A copy of that International Search Report is enclosed for the Examiner's convenience.

Five papers of possible interest here, at least four of which were published after the filings of both Wang and the present application, have come to counsel's attention and are noted here to complete the record and underscore that which has already been discussed. The first paper published is by Wang and her co-workers [Hosein et al., Proc. Natl. Acad. Sci. USA, 88:3647-3651 (May 1991)]. The second is by two of the present inventors and their co-workers [Sugitani et al., Lancet, 339:1018-1019 (April 1992)]. The third, by inventors herein [Nasoff et al., Proc. Natl. Acad. Sci. USA, 88:5462-66 (1991)] was published prior to the Wang paper. The fourth paper is Okamoto et al., Japan. J. Exp. Med., 60:222-233 (1990), whereas the fifth is Okamoto et al., Hepatology, 15:180-186 (1992).

The first paper (BA) discusses assays run using chemically synthesized peptides. An unidentified capsid (core) peptide "selected from a region covered by amino acids 1-120" was used as the single antigen in EIA I, peptides from two non-structural proteins were used in EIA II and all three peptides were used in EIA III. These three formats are thus similar to Formats A, B and C of the Wang patent.

Although there is not an exact identity of data (presumed to be due to the typographical errors because of the complete identity of the remaining data), it is believed that the data of Table 1 of this paper for donor 1 are the same as those of Table 8 of the Wang patent for panel 1. Similarly, the results of the second paragraph on the left side of page 3649 for

Japanese dialysis patients can be obtained by ready calculation from the data of the Wang patent Table 9. That being the case, the peptides of EIA II correspond to those of Format A of the Wang patent, whereas the EIA III peptides are those of Format C of the patent that used peptides IIH, V and VIIIE. Inasmuch as EIA III is said in the paper to contain all three peptides of EIA I and EIA II, the peptide of EIA I must have been peptide VIIIE of the patent.

This paper discusses the added sensitivity of anti-HCV antibody detection when a capsid synthetic peptide is added to peptides from non-structural proteins, including earlier detection of seroconversion as compared to the C-100 antigen-based assays. Missing, however, are data for the capsid synthetic peptide alone; i.e., peptide VIIIE.

The second paper (BB) compares various assays that include a Wang group kit (UBI-HCV, reference 5) an assay of the present invention (Capsid) and C100 kit used for comparison herein (C100-3). The data of the table show that an assay of the present invention based on a recombinant capsid corresponding to residues 1-120 was equally sensitive to the UBI-HCV kit containing three peptides and a second generation kit from Abbott (Abbott-II) that contains two non-structural antigens and a capsid antigen. All three identified 13/19 or 68 percent of the PCR-positive sera.

Thus, another unexpected result is found here. An assay of the claims based on a single recombinant whole protein (Fig. 1., residues 1-120) was as sensitive as an assay based on a mixture of three chemically synthesized peptides from three different proteins.

Enclosed paper three (BC) describes the CAP-N antigen used in the present application. Although the nomenclature is

different, it is apparent that the capsid antigen designated CAP-A of BB is the CAP-N antigen of the present application.

Document BD is an apparent follow-up to the Okamoto et al. paper of record herein that is cited twice in the paragraph bridging pages 1 and 2 of the present application. This paper deals with the use of a 36-mer synthetic peptide that contains residues 39-74 of the HCV capsid as an antigen in an assay for anti-HCV antibodies.

The first page of the article indicates that it was received for publication on June 13, 1993. A computer-assisted search in the MEDLINE data base of DIALOG Information Services, Inc., indicates that Document BD was published in August of 1990. The mailing and receipt dates of this article are unknown, but are being sought from counsel's Japanese associates and will be provided to the Examiner on receipt.

As is seen from the Summary, the anti-synthetic peptide assay (anti-CP9) and the commercial anti-HCV assay overlapped with positive results in 54 percent of 324 cases of acute or chronic NANB liver disease, with 18 percent of the sera being positive only in the anti-CP9 assay and another 15 percent of the sera being positive in the anti-HCV assay and negative in the anti-CP9 assay, leaving another 13 percent undetected in either assay.

Document BE published in 1992 is an apparent follow-up to Document BD. Here, another synthetic peptide was used in the assays. That peptide was designated CP10, and includes 19 residues covering amino acid residue positions 5-23 of Fig. 1. It is noted that this paper used the two peptides separately and summed the results obtained from separate assays rather than linking the peptides or using a mixture of both in the assays.

In accordance with 37 C.F.R. §1.98(2)(d), a copy of each of the listed documents was included with the Information Disclosure Statement filed with the grandparent application on April 10, 1992 and can be found in that application file.

Pursuant to 37 C.F.R. 1.98(d), it is understood that only a list of art is required inasmuch as the art has been provided and discussed previously.

No inferences should be drawn that the attached list represent a comprehensive investigation, or that any material disclosed is equivalent to the subject invention. In addition, none of the documents that have publication dates prior to the priority date of the above application anticipate the invention in this application.

The cited documents disclose numerous specific features. There has been no attempt to list each and every feature disclosed by each document. The Examiner is requested to review the documents and determine the extent of the materiality of the document disclosures with respect to the present invention.

The discussion of any art and the citation of any document herein is not to be construed as an admission that the art or document disclosure is necessarily within the invention field of endeavor, that the art or document disclosure is necessarily prior in time to a particular date which may be relevant to the instant patent application, and/or that the art or document disclosure is otherwise necessarily prior art as defined by the patent law with respect to the instant invention and application.

Also, there is reserved the right to later set forth how the instant invention is distinguished over the disclosure of any document or other art, including the disclosures of the art


Serial No. 08/272,275

-6-

and documents recited herein, that may be cited by the Examiner in rejecting a claim in the instant patent application.

The recitation herein of the art and documents is not to be construed as an assertion that more pertinent art could not possibly be in existence.

Respectfully submitted,

By 
Edward P. Gamson, Reg. No. 29,381

Enclosures:

Three (3) Forms PTO-1449
Copy of the International Search Report for
PCT Application PCT/US91/06037, which
corresponds to U.S. Patent Application
Serial No. 07/748,564

WELSH & KATZ, LTD.
135 South La Salle Street
Chicago, Illinois 60603
312/781-9470

CERTIFICATE OF MAILING

I hereby certify that this Information Disclosure Statement, together with the stated enclosures, is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Hon. Commissioner of Patents and Trademarks, Washington, D.C. 20231 on October 7, 1994.



U.S. Department of Commerce
Patent and Trademark Office

Atty. Docket No.
PNA-0025P CON I

Serial No.
08/272,275

Applicant
Zebedee et al.

INFORMATION DISCLOSURE CITATION
(Use several sheets if necessary)

Filing Date
July 8, 1994

Group
Not yet assigned

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

[illegible]

Examiner

Date Considered

*Examiner:

Initial if citation considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

U.S. Department of Commerce
Patent and Trademark Office

Alty. Docket No.
PHA-0025P CON I

Serial No.
08/272,275

Applicant
Zebedee et al.

INFORMATION DISCLOSURE CITATION
(Use several sheets if necessary)

Filing Date
July 8: 1994

Group
Not yet assigned

U.S. PATENT DOCUMENTS

[illegible]

FOREIGN PATENT DOCUMENTS

[illegible]

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

D	Choo et al, <u>Science</u> , <u>244</u> , 359-362 (1989)
E	Okamoto et al, <u>Japan J. Exp. Med.</u> , <u>60</u> , 163-177 (1990)
F	Miller et al., <u>PNAS</u> <u>87</u> , 2057-2061 (1990)
G	Kuo et al, <u>Science</u> <u>244</u>, 362-364 (1989)
H	Alter et al, <u>NEJM</u> <u>321</u> , 1538-39 (1989)

Examiner

Date Considered

*Examiner: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

(Rev. 5/92) Comparable to Form PTO-1449		U.S. Department of Commerce Patent and Trademark Office		Atty. Docket No. PHA-0025P CON I	Serial No. 08/272,275		
INFORMATION DISCLOSURE CITATION (Use several sheets if necessary)				Applicant Zebedee et al.			
				Filing Date July 8, 1994	Group Not yet assigned		
OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)							
I	Alter et al, <u>NEJM</u> , <u>321</u> , 1494-1500 (1989)						
J	Weiner et al, <u>Lancet</u> , <u>335</u> , 1-3 (1990)						
K	McFarlane et al, <u>Lancet</u> , <u>335</u> , 754-757 (1990)						
L	Grey et al, <u>Lancet</u> , <u>335</u> , 609-610 (1990)						
M	Houghton et al, <u>Int. K. Prot Res</u> , <u>16</u> , 311-320 (1980)						
O	Choo et al, <u>PNAS</u> , <u>88</u> 2451-2455 (1991)						
P	Takamizawa et al., <u>J. Virol.</u> , <u>65</u> , 1105-1113 (1991)						
Q	Kato et al, <u>PNAS</u> , <u>87</u> , 9524-9528 (1990)						
R	Takeuchi et al, <u>Nucleic Acids Res.</u>, <u>18</u>, 4626 (1990)						
S	Ogata et al, <u>PNAS</u> , <u>88</u> , 3392-3396 (1991)						
T	Ilan et al., <u>PNAS</u> , <u>88</u> , 1711-1715 (1991)						
U	Meyer et al., <u>Virol</u> , <u>171</u> , 555-567 (1989)						
V	Collett et al., <u>Virol</u> , <u>165</u> , 191-199 (1988)						
W	Brinton et al., <u>Virol</u> , <u>162</u> , 290-299 (1988)						
X	Inchauspe et al, <u>PNAS</u> , <u>88</u> , 10292-10296 (1991)						
Y	Wiener et al, <u>Virol</u> , <u>180</u> , 842-848 (1991)						
Z	Hahn et al, <u>Virol</u> , <u>162</u> , 167-180 (1988)						
AA	Prince et al, <u>Lancet</u> , 2:241 (1974)						
AB	Prince et al., "Posttransfusion Viral Hepatitis Caused by an Agent or Agents Other Than Hepatitis B Virus or Hepatitis A Virus. Impact On Efficiency of Present Screening Methods." in <u>Transmissible Disease & Blood Transfusion</u> , Tibor et al. eds., Grune & Stratton, Inc., pp. 129-140 (1975)						
AC	Prince et al., "Non-A/Non-B Hepatitis: Identification of a virus-specific antigen and antibody. A preliminary report" in <u>Viral Hepatitis</u> , Vyas et al., eds., Franklin Institute Press, Philadelphia, Pa. pp. 633-640 (1978).						
AD	Prince et al., "Non-A, Non-B Hepatitis: Reproduction of disease in chimpanzees and identification of virus specific antigen and antibody" in <u>Transplantation and Clinical Immunology</u> , Volume X, Touraine et al., eds., Excerpta Medica, Amsterdam, pp. 8-17 (1979).						
AE	Prince, A.M., <u>Lancet</u> , May 22, 1982.						
Examiner <i>Norma C. [Signature]</i>		Date Considered <i>12/8/94</i>					
*Examiner: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.							

U.S. Department of Commerce
Patent and Trademark Office

Atty. Docket No.
PHA-0025P CON I

Serial No.
08/272,275

INFORMATION DISCLOSURE CITATION
(Use several sheets if necessary)

Applicant
Zebedee et al.

Filing Date
July 8; 1994

Group
Not yet assigned

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

AF Prince et al., "Use of liver cell cultures in studies on the replication of hepadna and non-A, non-B viruses" in Viral Hepatitis and Liver Disease, Grune & Stratton, pp. 459-466 (1984).

AG Brotman et al., J. Infect. Diseases, 151(4):618 (1985)

AH Takeuchi, et al. Gene, 91:287 (1990)

Examiner

Date Considered 12/8/94

*Examiner: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

08/27275


**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

 Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/272,275	07/08/94	ZEBEDEE	S

 WELSH & KATZ LTD
SUITE 1625
135 SOUTH LA SALLE STREET
CHICAGO IL 60603

18N1/1216

WORTMAN EXAMINER

ART UNIT	PAPER NUMBER
1802	31

1802

DATE MAILED: 12/16/94

 This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined. ☐ Responsive to communication filed on _____ ☐ This action is made final.

 A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133
Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- | | |
|---|---|
| 1. <input checked="" type="checkbox"/> Notice of References Cited by Examiner, PTO-892. | 2. <input type="checkbox"/> Notice of Draftsman's Patent Drawing Review, PTO-948. |
| 3. <input checked="" type="checkbox"/> Notice of Art Cited by Applicant, PTO-1449. | 4. <input type="checkbox"/> Notice of Informal Patent Application, PTO-152. |
| 5. <input type="checkbox"/> Information on How to Effect Drawing Changes, PTO-1474. | 6. <input type="checkbox"/> _____ |

Part II SUMMARY OF ACTION

- 1.
- ☒
- Claims
- 28, 30-37
- are pending in the application.

Of the above, claims _____ are withdrawn from consideration.

- 2.
- ☒
- Claims
- 1-27, 29, 38-40
- have been cancelled.

- 3.
- ☐
- Claims _____ are allowed.

- 4.
- ☒
- Claims
- 28, 30-37
- are rejected.

- 5.
- ☐
- Claims _____ are objected to.

- 6.
- ☐
- Claims _____ are subject to restriction or election requirement.

- 7.
- ☐
- This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

- 8.
- ☐
- Formal drawings are required in response to this Office action.

- 9.
- ☐
- The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are
- ☐
- acceptable;
- ☐
- not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).

- 10.
- ☐
- The proposed additional or substitute sheet(s) of drawings, filed on _____, has (have) been:
- ☐
- approved by the examiner;
- ☐
- disapproved by the examiner (see explanation).

- 11.
- ☐
- The proposed drawing correction, filed _____, has been:
- ☐
- approved;
- ☐
- disapproved (see explanation).

- 12.
- ☐
- Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has
- ☐
- been received;
- ☐
- not been received.
- ☐
- been filed in parent application, serial no. _____; filed on _____.

- 13.
- ☐
- Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

- 14.
- ☐
- Other _____

EXAMINER'S ACTION

Serial Number: 08/272275

-2-

Art Unit: 1802

Claim 29 was cancelled and Claim 28 was amended by the Preliminary Amendment filed as Paper No. 29. Claims 1-27 had been previously cancelled. Consequently Claims 28 and 30-37 are pending and under examination at this time.

Claims 28 and 30-37 are rejected under 35 U.S.C. 12, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 28 is indefinite because it recites "said recombinant protein or portion thereof having the amino acid sequence represented by ... or ..." and it is not immediately clear what protein or portion thereof the sequences recited are intended to represent. From Applicant's remarks it is believed that the specific sequence from Fig. 1 is intended to be the recited "protein" and the specific sequence from Fig. 2 is intended to be the "portion thereof." If such is the case, it is suggested that the language be amended to clarify, e.g., "wherein said recombinant protein has the sequence ... and said portion has the sequence ...".

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 02 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --
(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(c) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section

Serial Number: 08/272275

-3-

Art Unit: 1802

371(c) of this title before the invention thereof by the applicant for patent.

The following is a quotation of 35 U.S.C. 03 which forms the basis for all obviousness rejections set forth in this Office action:

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 03, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 C.F.R. 56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. 02(f) or (g) prior art under 35 U.S.C. 03.

Claims 28 and 30-37 are rejected under 35 U.S.C. 03 as obvious over the patent to Wang in view of Kuo. Wang teaches assaying sera for antibodies against HCV (NANBV) using solid phase coated with synthetic peptides that include amino acid residue sequences from the HCV core (see Wang, Example 14 and Table 7, especially peptides VIIIE, IXD, and IXE). The recited sequences would have been obvious over Wang because there are only slight differences between them and the core sequences taught by Wang. Wang does not exemplify producing recombinant HCV polypeptides, but suggests that recombinant antigens may be substituted for synthetic antigens: Wang, col. 25, lines 29-42.

Serial Number: 08/272275

-4-

Art Unit: 1802

Kuo teaches production of an HCV recombinant fusion protein and detection of serum antibodies using the recombinant fusion protein. It would have been obvious to one of ordinary skill in the art to produce the HCV peptide of Wang recombinantly as taught by Kuo and as suggested by Wang in order to gain the advantages of producing peptides by recombinant means, e.g., to obtain a stable, plentiful supply of peptides that are free of contamination with other HCV antigens. One of ordinary skill in the art at the time the invention was made would have recognized that there are advantages to using recombinant antigens and if one were willing to forego the advantages of synthetics in favor of the known advantages of recombinants, then one would have been motivated to select recombinant antigens.

Applicant's previous arguments referring to Wang's results as compared to C100 and the instant antigen's results compared to C100 have not been found persuasive for the reasons made of record in the Advisory Action, Paper No. 25.

Claim 28 is rejected under 35 U.S.C. 02(e) as anticipated by or, in the alternative, under 35 U.S.C. 03 as obvious over the patent to Houghton et al., cited on PTO 892, attached. It is believed that Houghton anticipates an immunoassay using as recombinant antigen the sequence represented in Fig. 1, amino acid residues 1-120 (see, e.g., col. 81, lines 55-65), but if not, any minor differences in the antigen sequence 1-120 as taught by Houghton are no more than one would have expected to occur from one HCV isolate to another.

Serial Number: 08/272275

-5-

Art Unit: 1802

Claim 28 is rejected under 35 U.S.C. 03 as being unpatentable over the patent to Houghton et al., as cited on PTO 892. An immunoassay using as antigen the sequence represented by Fig. 2, amino acid residues 1-315, which includes HCV core antigen sequence 1-74, would have been an obvious variant of Houghton's disclosed recombinant antigen CA279a which contains HCV core antigen 1-84 (see, e.g., col. 83, lines 35-55) because HCV 1-74 would have nearly 90% of the amino acids present in 1-84 and the first 74 amino acids are the same, allowing for minor variations which may occur from one viral isolate to another. Consequently, one of ordinary skill would have expected an antigen containing core sequence 1-74 to function in an essentially equivalent fashion to one with the HCV core sequence 1-84.

Claims 30-37 are rejected under 35 U.S.C. 03 as obvious over the patent to Houghton et al. Houghton teaches use of HCV core antigens as discussed above and additionally teaches different assay formats and labels. Thus it would have been obvious to use the HCV core antigens as discussed above with various formats and labels because Houghton suggests these as being useful with any HCV antigen.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Chiba et al., US Patent No. 5,302,507, teach an HCV antigenic peptide consisting of HCV core amino acids 1-20.

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform to the notice published in the

Serial Number: 08/272275

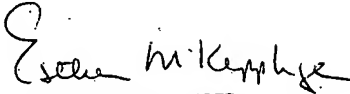
-6-

Art Unit: 1802

Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax
Center number is (703) 308-4227.

Any inquiry concerning this communication should be directed
to Examiner Donna C. Wortman at telephone number (703) 308-1032.

Donna C. Wortman, Ph.D.
December 13, 1994


ESTHER M. KEPLINGER
SUPERVISORY PATENT EXAMINER
GROUP 1800

[illegible]

\$870.00 - 117

Gr. 1802
32
LP
21845



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee et al.)
File-Wrapper Continuation of) Attorney Docket
Serial No.: 08/272,275) PHA 0025P CON II
) (2673/63066)
Filed: June 14, 1995)
For: NON-A, NON-B, HEPATITIS VIRUS) Prior Group Art
ANTIGEN, DIAGNOSTIC METHODS AND) Unit 1802
VACCINES)
Prior Examiner: D. Wortman)

PETITION UNDER 37 C.F.R. §1.17

JUL 13 1995

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

A three-month extension of time to respond to the Office Action mailed December 16, 1994, is respectfully requested. This Petition is requested in order to allow the applicants to file a Rule 1.62 continuation application, enclosed herewith.

There is submitted herewith the following:


1. Check No. 027823 in the amount of the required fee of \$870.00 for the three-month extension of time (a response to the Office Action was due March 16, 1995);
2. Check No. 027822 in the amount of \$730.00 for the filing fee for the continuation application; and
3. Transmittal letter under Rule 1.62, in duplicate, for the continuation application.

Adm. cont. to A

File-Wrapper Continuation of , -2-
Serial No. 08/272,275

The Commissioner is hereby authorized to charge payment
of any additional fees or credit any overpayment to Deposit
Account No. 04-1644. A duplicate copy of this paper is enclosed.

Respectfully submitted,

By 
Edward P. Gamson, Reg. No. 29,381

Enclosure

Transmittal for Continuation Application and fee
fee for Extension of Time

WELSH & KATZ, LTD.
135 South La Salle Street
Chicago, Illinois 60603
312/781-9470

CERTIFICATE OF MAILING BY EXPRESS MAIL

I hereby certify that this Petition for Three-Month
Extension of Time, Transmittal for continuation Application in
duplicate, together with the aforementioned documents and the
required fees, is being deposited as Express Mail No.
TB039739403US on June 14, 1995, addressed to Box FWC, Assistant
Commissioner for Patents, Washington, D.C. 20231.



PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 1992

Application or Docket Number

08/272275

CLAIMS AS FILED - PART I

(Column 1)

(Column 2)

SMALL ENTITY

OR

**OTHER THAN
SMALL ENTITY**

FOR	NUMBER FILED	NUMBER EXTRA
BASIC FEE		
TOTAL CLAIMS	9 minus 20 = *	
INDEPENDENT CLAIMS	1 minus 3 = *	
MULTIPLE DEPENDENT CLAIM PRESENT		

RATE	FEE
	\$355.00
x \$11=	
x 37=	
+115=	
TOTAL	

RATE	FEE
	\$710.00
x \$22=	
x 74=	
+230=	
TOTAL	710

* If the difference in column 1 is less than zero, enter "0" in column 2.

CLAIMS AS AMENDED - PART II

(Column 1)

(Column 2)

(Column 3)

SMALL ENTITY

OR

**OTHER THAN
SMALL ENTITY**

AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	*	Minus **	=
Independent	*	Minus ***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

RATE	ADDI- TIONAL FEE
x \$11=	
x 37=	
+ 115=	
TOTAL	
ADDIT. FEE	

RATE	ADDI- TIONAL FEE
x \$22=	
x 74=	
+230=	
TOTAL	
ADDIT. FEE	

AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	*	Minus **	=
Independent	*	Minus ***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

RATE	ADDI- TIONAL FEE
x \$11=	
x 37=	
+ 115=	
TOTAL	
ADDIT. FEE	

RATE	ADDI- TIONAL FEE
x \$22=	
x 74=	
+ 230=	
TOTAL	
ADDIT. FEE	

AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	*	Minus **	=
Independent	*	Minus ***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

RATE	ADDI- TIONAL FEE
x \$11=	
x 37=	
+115=	
TOTAL	
ADDIT. FEE	

RATE	ADDI- TIONAL FEE
x \$22=	
x 74=	
+230=	
TOTAL	
ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

DATE 8-2-94

**SHEETS OF
DRAWING**

ATTORNEY DOCKET NUMBER

[illegible]

PARENTS WILLING

PARENT FILING DATE		
MONTH	DAY	YEAR
0		

0	8	2	7	9	0
---	---	---	---	---	---

**FOREIGN
FILING DATE**

MONTH	DAY	YEAR
12	15	2000

III. A check in the amount of \$730.00 is enclosed herewith for the filing fee. The fee is calculated on the basis of the 9 total claims, including 1 independent claim pursuant to the claims as filed and last amended in the prior application.

IV.

Fee Calculation For Claims As Filed

a)	Basic Fee					\$ 730.00
b)	Independent Claims	<u>1</u>	-	<u>3</u>	=	<u>0</u> x \$ 76.00 = \$ <u>0</u>
c)	Total Claims	<u>9</u>	-	<u>20</u>	=	<u>0</u> x \$ 22.00 = \$ <u>0</u>
d)	Fee for Multiple Claims					\$ 240.00 = \$ <u>0.00</u>
	Total Filing Fee					\$ <u>730.00</u>

Small Entity Status, reducing Filing Fee by half to \$

V. It is understood that the filing of this Request will be considered to include a request to expressly abandon the prior application as of the filing date granted this continuing application.

VI. The pertinent information, as required by 37 CFR §1.62(h) is as follows:

Title: NON-A, NON-B, HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

Applicants: Suzanne Zebedee
Genevieve Inchausepe
Marc Nasoff
Alfred Prince

VII. The title of the invention in the continuing application should be NON-A, NON-B, HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES and the

inventors to be named in the continuing application are Suzanne Zebedee, Genevieve Inchausepe, Marc Nasoff and Alfred Prince.

- VIII. Amend the specification by inserting before the first line the sentence:

DI -- This application is a continuation, of application Serial No. 08/272,275, filed July 8, 1994. --

- IX. The prior application is assigned of record to:
The New York Blood Center
- X. The power of attorney in the prior application is to:
Edward P. Gamson, Esq., Reg. 29,381
- XI. The Commissioner is authorized to charge any additional fees, and to credit any refunds, to Deposit Account No. 23-0920. A duplicate of this Request is enclosed.


Please address all future correspondence to:

Edward P. Gamson, Esq.
Welsh & Katz, Ltd.
135 South LaSalle Street
Suite 1625
Chicago, Illinois 60603
(312) 781-9470

Respectfully submitted,

WELSH & KATZ, LTD.

By:


Edward P. Gamson, Esq.
Registration No. 29,381
Attorney for Applicants

June 14, 1995

Welsh & Katz, Ltd.
135 South LaSalle Street
Suite 1625
Chicago, Illinois 60603
Tel: (312) 781-9470

1434
100
PATENT 5/29/96

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Patent(s) / Patent Application(s)

Applicant:

Serial No.: 08/489,254 Wortman

Filed: 1813

Art Unit:

Examiner:

RECEPTIONIST
RECEIVED

MAR 27 1996

GROUP 1800

GENERAL POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

The undersigned attorney of record in each of the patents and patent applications identified in the attached seven (7) pages relating to the change of address of Welsh & Katz, Ltd. for each application hereby appoints Peter Borsari, Registration No. 32,114_ as associate attorney in each of the applications listed in the attached seven pages to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith.

Dated: February 28, 1996

Gerald T. Shekleton
Gerald T. Shekleton
Registration No. 27,466

WELSH & KATZ, LTD.
120 South Riverside Plaza
22nd Floor
Chicago, Illinois 60606-3913
Telephone: 312/655-1500

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner of Patents
Box Patent Address Change
Washington D.C. 20231

CHANGE OF ATTORNEYS' MAILING ADDRESS

Dear Sir:

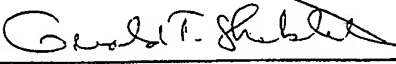
In the below-listed Patent applications, please make the following address change for the attorneys of record:

Welsh & Katz, Ltd.
120 South Riverside Plaza
22nd Floor
Chicago, IL 60606
Phone: (312) 655-1500
Fax: (312) 655-1501

<u>Appln. No.</u>	<u>First Named Inventor</u>	<u>Appln. Nos.</u>	<u>First Named Inventor</u>
044,094	RICE	472,146	AW
529,308	RICE	489,232	SLATER
415,623	ANDO-MODIANO	489,254	ZEBEDEE
409,178	POZZEBON	524,351	NELSON
416,999	WONG	531,193	NELSON
407,985	FURUYA	491,626	TABIBI
410,746	HALE	492,537	UEDA
408,944	SCHAKOWSKY	521,210	DATTA
415,120	WOJCIK	496,351	COHEN
037,931	GENTRY	496,935	TSAI
491,401	GENTRY	499,010	IKEO
441,379	BERNSTEIN	499,558	FOFFAN
472,708	SPANG	501,884	KARP
416,451	MURATA-KIMURA	503,530	DRAVID
441,123	LASKA	504,510	CHIEN
478,382	ROGERS	508,404	MOORE
530,811	VEAL	524,350	NELSON
240,496	WOLF	517,090	LASKA
423,266	NICOLAOU	448,117	CONCOSCENTI
517,172	RAZEGHI	425,352	MATSUNAGA
514,533	HUS	427,136	EDAUW
536,667	CARTER	436,981	TAKEBE
515,703	MURASKI	436,931	TAKEBE
532,239	CURTIS	442,640	KLUTZNIICK
519,617	IKEO	462,957	FORBES
524,160	HANDWERKER	508,292	WOOLLEY
531,237	WEISS	446,445	CHEN
044,136	HEBROCK	496,822	SCHEFFER
522,179	HABER	451,914	CHAPELON
521,841	TAKEBE	513,011	HEBROCK
522,912	YUKIMOTO	513,228	STARK
524,301	FOX	455,498	
524,043	WU	466,927	SUTCLIFFE
527,340	LENG	503,066	RAZEGHI
531,162	KREMNITZ	486,765	MOLNAR
532,942	HUNT	472,877	WONG
044,459	EDAUW	476,685	WONG
044,477	EDAUW	473,512	AUSICH
044,458	EDAUW	470,172	YOKOHAMA
044,455	EDAUW	198,553	PLAEHN
534,380	PIVA	508,099	HAMETTA
308,551	CHAPPELL		

The original of this document is being filed in the Enrollment and Discipline Office. It is certified that the person whose signature appears below has the authority to make the requested changes in the patent.

December 29, 1995


Gerald T. Shekleton
Reg. No. 27,466

08/489,254



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
--------------------	-------------	-----------------------	---------------------

08/489,254 06/14/95 ZEBEDEE

S 2673/63066

EXAMINER
WURTMAN, D

18M1/0917

EDWARD P GAMSON
WELSH AND KATZ
120 SOUTH RIVERSIDE PLAZA
22ND FLOOR
CHICAGO IL 60606

ART UNIT PAPER NUMBER

DATE MAILED:

09/17/96

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

- ☒ Responsive to communication(s) filed on 6/14/95
- ☐ This action is FINAL.

- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims.

- ☒ Claim(s) 28 and 30-37 is/are pending in the application.
- Of the above, claim(s) _____ is/are withdrawn from consideration.
- ☐ Claim(s) _____ is/are allowed.
- ☒ Claim(s) 28 and 30-37 is/are rejected.
- ☐ Claim(s) _____ is/are objected to.
- ☐ Claims _____ are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- ☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some ☐ None of the CERTIFIED copies of the priority documents have been
- ☐ received.
- ☐ received in Application No. (Series Code/Serial Number) _____
- ☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

- ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- ☐ Notice of Reference Cited, PTO-892
- ☐ Information Disclosure Statement(s), PTO-1449, Paper No(s) _____
- ☐ Interview Summary, PTO-413
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ Notice of Informal Patent Application, PTO-152.

SEE OFFICE ACTION ON THE FOLLOWING PAGES.

Serial Number: 08/489254

-2-

Art Unit: 1813

Claims 28 and 30-37 remain pending and under examination at this time.

Claims 28 and 30-37 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 28 is indefinite because it recites "said recombinant protein or portion thereof having the amino acid sequence represented by ... or ..." and it is not immediately clear what protein or portion thereof the sequences recited are intended to represent. From Applicant's remarks it is believed that the specific sequence from Fig. 1 is intended to be the recited "protein" and the specific sequence from Fig. 2 is intended to be the "portion thereof." If such is the case, it is suggested that the language be amended to clarify, e.g., "wherein said recombinant protein has the sequence ... and said portion has the sequence ...".

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by

Serial Number: 08/489254

-3-

Art Unit: 1813

the same person or subject to an obligation of assignment to the same person.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. § 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 C.F.R. § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. § 102(f) or (g) prior art under 35 U.S.C. § 103.

Claims 28 and 30-37 are rejected under 35 U.S.C. § 103 as obvious over patent 5,106,726 to Wang in view of Kuo et al., both of record. Wang teaches assaying sera for antibodies against HCV (NANBV) using solid phase coated with synthetic peptides that include amino acid residue sequences from the HCV core (see Wang, Example 14 and Table 7, especially peptides VIIIE, IXD, and IXE). The recited sequences would have been obvious over Wang because there are only slight differences between them and the core sequences taught by Wang. Wang does not exemplify producing recombinant HCV polypeptides, but suggests that recombinant antigens may be substituted for synthetic antigens: Wang, col. 25, lines 29-42. Kuo teaches production of an HCV recombinant fusion protein and detection of serum antibodies using the recombinant fusion protein. It would have been obvious to one of ordinary skill in the art to produce the HCV peptide of Wang recombinantly as taught by Kuo and as suggested by Wang in order to gain the advantages of producing peptides by recombinant means, e.g., to obtain a stable, plentiful supply of peptides that are free of contamination with other HCV antigens. One of ordinary skill in the art at the time the invention was made would have recognized that there are advantages to using recombinant antigens and if one were willing to forego the advantages of synthetics in favor of the known advantages of recombinants, then one would have been motivated to select recombinant antigens.

Applicant's previous arguments referring to Wang's results as compared to C100 and the instant antigen's results compared to C100 have not been found

Serial Number: 08/489254

-4-

Art Unit: 1813

persuasive for the reasons made of record in the Advisory Action, Paper No. 25.

Claim 28 is rejected under 35 U.S.C. § 102(e) as anticipated by or, in the alternative, under 35 U.S.C. § 103 as obvious over patent 5,350,671 to Houghton et al., of record. It is believed that Houghton anticipates an immunoassay using as recombinant antigen the sequence represented in Fig. 1, amino acid residues 1-120 (see, e.g., col. 81, lines 55-65), but if not, any minor differences in the antigen sequence 1-120 as taught by Houghton are no more than one would have expected to occur from one HCV isolate to another.

Claim 28 is rejected under 35 U.S.C. § 103 as being unpatentable over patent 5,350,671 to Houghton et al. An immunoassay using as antigen the sequence represented by Fig. 2, amino acid residues 1-315, which includes HCV core antigen sequence 1-74, would have been an obvious variant of Houghton's disclosed recombinant antigen CA279a which contains HCV core antigen 1-84 (see, e.g., col. 83, lines 35-55) because HCV 1-74 would have nearly 90% of the amino acids present in 1-84 and the first 74 amino acids are the same, allowing for minor variations which may occur from one viral isolate to another. Consequently, one of ordinary skill would have expected an antigen containing core sequence 1-74 to function in an essentially equivalent fashion to one with the HCV core sequence 1-84.

Claims 30-37 are rejected under 35 U.S.C. § 103 as obvious over the patent to Houghton et al. as cited and applied above. Houghton teaches use of HCV core antigens as discussed above and additionally teaches different assay formats and labels. Thus it would have been obvious to use the HCV core antigens as discussed above with various formats and labels because Houghton suggests these as being useful with any HCV antigen.

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform to

Serial Number: 08/489254

-5-

Art Unit: 1813

the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989).

The CMI Fax Center number is (703) 308-4227.

Any inquiry concerning this communication should be directed to Examiner
Donna C. Wortman at telephone number (703) 308-1032.

Donna C. Wortman, Ph.D.
September 16, 1996

Mary E. Mosher
MARY E. MOSHER
PRIMARY EXAMINER
GROUP 1800



930-117

35 1/2
5/21/97

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Zebedee et al.

File-Wrapper Continuation of
Serial No.: 08/272,275

Filed: June 14, 1995

For: NON-A, NON-B, HEPATITIS VIRUS
ANTIGEN, DIAGNOSTIC METHODS AND
VACCINES

Prior Examiner: D. Wortman

)
) Attorney Docket
) PHA 0025P CON
) (2673/63066)
)
)
) Prior Group Art
) Unit 1802
)
)
)
)
)260 NJ 04/02/97 082734
1 117 930.00 EXPETITION UNDER 37 C.F.R. §1.17Assistant Commissioner for Patents
Washington, D.C. 20231RECEIVED
APR 04 1997
GROUP 1800

Sir:

A three-month extension of time to respond to the Office Action mailed September 17, 1996, is respectfully requested. This Petition is requested in order to allow the applicants to file a Rule 1.62 continuation application, enclosed herewith.

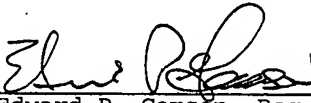
There is submitted herewith the following:

1. Check No. 036717 in the amount of the required fee of \$930.00 for the three-month extension of time (a response to the Office Action was due December 17, 1996);
2. Check No. 036716 in the amount of \$770.00 for the filing fee for the continuation application; and
3. Transmittal letter under Rule 1.62, in duplicate, for the continuation application.

File-Wrapper Continuation of -2-
Serial No. 08/272,275

The Commissioner is hereby authorized to charge payment
of any additional fees or credit any overpayment to Deposit
Account No. 04-1644. A duplicate copy of this paper is enclosed.

Respectfully submitted,

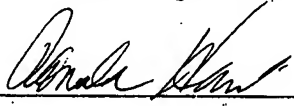
By 
Edward P. Gamson, Reg. No. 29,381

Enclosure
Transmittal for Rule 1.62 Continuation Application and fee
Fee for Extension of Time

WELSH & KATZ, LTD.
120 South Riverside Plaza
22nd Floor
Chicago, Illinois 60606
312/655-1500

CERTIFICATE OF MAILING BY EXPRESS MAIL

I hereby certify that this Petition for Three-Month
Extension of Time, Transmittal for Rule 1.62 Continuation
Application in duplicate, together with the aforementioned
documents and the required fees, is being deposited as Express
Mail No. EM509798657US on March 17, 1997, addressed to Box FWC,
Assistant Commissioner for Patents, Washington, D.C. 20231.



CSH89254



**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/489,254	06/14/95	ZEBEDEE	S 2673/63066

EDWARD P. GAMSON
WELSH AND KATZ
120 SOUTH RIVERSIDE PLAZA
22ND FLOOR
CHICAGO IL 60606

18M1/0513

EXAMINER	
WORTMAN, D	
ART UNIT	PAPER NUMBER
1815	36

DATE MAILED: 05/13/97

NOTICE OF ABANDONMENT

This application is abandoned in view of:

1. ☒ Applicant's failure to respond to the Office letter, mailed 9/17/96.
2. ☐ Applicant's letter of express abandonment which is in compliance with 37 C.F.R. 1.138.
3. ☐ Applicant's failure to timely file the response received _____ within the period set in the Office letter.
4. ☐ Applicant's failure to pay the required issue fee within the statutory period of 3 months from the mailing date of _____ of the Notice of Allowance.
 - ☐ The issue fee was received on _____.
 - ☐ The issue fee has not been received in Allowed Files Branch as of _____.

In accordance with 35 U.S.C. 151, and under the provisions of 37 C.F.R. 1.316(b), applicant(s) may petition the Commissioner to accept the delayed payment of the issue fee if the delay in payment was unavoidable. The petition must be accompanied by the issue fee, unless it has been previously submitted, in the amount specified by 37 C.F.R. 1.17 (l), and a verified showing as to the causes of the delay.

If applicant(s) never received the Notice of Allowance, a petition for a new Notice of Allowance and withdrawal of the holding of abandonment may be appropriate in view of *Delgar Inc. v. Schuyler*, 172 U.S.P.Q. 513.

5. ☐ Applicant's failure to timely correct the drawings and/or submit new or substitute formal drawings by _____ as required in the last Office action.
 - ☐ The corrected and/or substitute drawings were received on _____.
6. ☐ The reason(s) below.

Marian C. Knode

Ben

MARIAN C. KNODE
SUPERVISORY PATENT EXAMINER
GROUP 1800

MAIL ROOM
MAR 17 1991
EXP. PAT. & TRADEMARK OFF.

PATENT APPLICATION

Patent Application of
Suzanne Zebedee
Genevieve Inchausepe
Marc Nasoff
Alfred Prince

Est. 535-11
Go, 802

III. A check in the amount of \$730.00 is enclosed herewith for the filing fee. The fee is calculated on the basis of the 9 total claims, including 1 independent claim pursuant to the claims as filed and last amended in the prior application.

IV.

Fee Calculation For Claims As Filed

a)	Basic Fee					\$ 770.00
b)	Independent Claims	<u>1</u>	-	3	=	<u>0</u> x \$ 80.00 = \$ <u>0</u>
c)	Total Claims	<u>9</u>	-	20	=	<u>0</u> x \$ 22.00 = \$ <u>0</u>
d)	Multiple Dependent Claims					\$ 260.00 = \$ <u>0.00</u>
	Total Filing Fee					\$ <u>770.00</u>

Small Entity Status, reducing Filing Fee by half to \$

V. It is understood that the filing of this Request will be considered to include a request to expressly abandon the prior application as of the filing date granted this continuing application.

VI. The pertinent information, as required by 37 CFR §1.62(h) is as follows:

Title: NON-A, NON-B, HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

Applicants: Suzanne Zebedee
Genevieve Inchausepe
Marc Nasoff
Alfred Prince

VII. The title of the invention in the continuing application should be NON-A, NON-B, HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES and the

inventors to be named in the continuing application are Suzanne Zebedee, Genevieve Inchausepe, Marc Nasoff and Alfred Prince.

VIII. Amend the specification by inserting before the first line the sentence:

E1 -- This application is a continuation, of application Serial No. 08/272,275, filed July 8, 1994. --

IX. The prior application is assigned of record to:

The New York Blood Center

X. The power of attorney in the prior application is to:

Edward P. Gamson, Esq., Reg. 29,381

XI. The Commissioner is authorized to charge any additional fees, and to credit any refunds, to Deposit Account No. 23-0920. A duplicate of this Request is enclosed.

Please address all future correspondence to:

Edward P. Gamson, Esq.
~~Welsh & Katz, Ltd.~~
~~120 South Riverside Plaza~~
~~22nd Floor~~
~~Chicago, Illinois 60606~~
~~(312) 655-1500~~

Respectfully submitted,

WELSH & KATZ, LTD.

By:

Edward P. Gamson
Edward P. Gamson, Esq. (C)
Registration No. 29,381
Attorney for Applicants

March 17, 1997

Welsh & Katz, Ltd.
120 South Riverside Plaza
22nd Floor
Chicago, Illinois 60606
Tel: (312) 655-1500

PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 1994

Application or Docket Number

08/489254

CLAIMS AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA
BASIC FEE		
TOTAL CLAIMS	9 minus 20 =	0
INDEPENDENT CLAIMS	1 minus 3 =	0
MULTIPLE DEPENDENT CLAIM PRESENT		

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
	365.00	OR		730.00
x\$11=		OR	x\$22=	
x38=		OR	x76=	
+120=		OR	+240=	
TOTAL		OR	TOTAL	730.00

CLAIMS AS AMENDED - PART II

AMENDMENT A	(Column 1)	(Column 2)	(Column 3)	
	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	
	Total	Minus	**	=
	Independent	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=		OR	x\$22=	
x38=		OR	x76=	
+120=		OR	+240=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B	(Column 1)	(Column 2)	(Column 3)	
	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	
	Total	Minus	**	=
	Independent	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=		OR	x\$22=	
x38=		OR	x76=	
+120=		OR	+240=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C	(Column 1)	(Column 2)	(Column 3)	
	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	
	Total	Minus	**	=
	Independent	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=		OR	x\$22=	
x38=		OR	x76=	
+120=		OR	+240=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
 ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
 *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
 The Highest Number Previously Paid For (Total or Independent) is the highest number found in the appropriate box in column 1.

**SHEETS OF
DRAWING**

• •

3	1	6	3	6	6	6	6
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DATE	DAY	MONTH
10/10/10	10	10

[illegible]

PATENT APPLICATION SERIAL NO. 08/819857

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

290 LC.04/10/97 08019857 ✓
1 101 774.00 (x 25/300-02)

BAR CODE LABEL							
		U.S. PATENT APPLICATION					
SERIAL NUMBER	FILING DATE	CLASS		GROUP ART UNIT			
08/819,857	03/17/97	435		1802			
APPLICANT	SUZANNE ZEBEDEE, SAN DIEGO, CA; GENEVIEVE INCHAUSEPE, NEW YORK, NY; MARC S. NASOFF, SAIN DIEGO, CA; ALFRED M. PRINCE, NEW YORK, NY.						
	CONTINUING DATA*** VERIFIED THIS APPLN IS A CON OF 08/272,275 07/08/94 WHICH IS A CON OF 07/573,643 08/27/90						
	FOREIGN/PCT APPLICATIONS*** VERIFIED						
FOREIGN FILING LICENSE GRANTED 05/01/97							
STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS	FILING FEE RECEIVED	ATTORNEY DOCKET NO.		
CA	4	9	1	\$770.00	2673/63066		
ADDRESS	EDWARD P GAMSON WELSH AND KATZ LTD 120 SOUTH RIVERSIDE PLAZA 22ND FLOOR CHICAGO IL 60606						
TITLE	NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES						
This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application which is identified above. By authority of the COMMISSIONER OF PATENTS AND TRADEMARKS							
Date	Certifying Officer						



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, DC 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/819,857	03/17/97	ZEBEDEE	S 2673/63066

EDWARD P. GAMSON
WELSH AND KATZ LTD
22ND FLOOR
120 SOUTH RIVERSIDE PLAZA
CHICAGO IL 60606

HM21/0928

EXAMINER

WORTMAN, D

ART UNIT

PAPER NUMBER

1643

38

DATE MAILED:

09/28/98

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action SummaryApplication No.
08/819,857Applicant(s)
Zebedee et al.Examiner
Donna C. Wortman, Ph.D.Group Art Unit
1643☒ Responsive to communication(s) filed on Mar 17, 1997☐ This action is FINAL.☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims☒ Claim(s) 28 and 30-37 is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.☒ Claim(s) 28 and 30-37 is/are rejected.☐ Claim(s) _____ is/are objected to.☐ Claims _____ are subject to restriction or election requirement.**Application Papers**☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.☐ The drawing(s) filed on _____ is/are objected to by the Examiner.☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.☐ The specification is objected to by the Examiner.☐ The oath or declaration is objected to by the Examiner.**Priority under 35 U.S.C. § 119**☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been
☐ received.☐ received in Application No. (Series Code/Serial Number) _____☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).**Attachment(s)**☒ Notice of References Cited, PTO-892☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____☐ Interview Summary, PTO-413☐ Notice of Draftsperson's Patent Drawing Review, PTO-948☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

Application/Control Number: 08/819857

Page 2

Art Unit: 1643

The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1643.

This application is a file wrapper continuation of application Serial No. 08/489254. Claims 28 and 30-37 remain pending and under examination at this time.

Applicant is requested to update and correct the continuing information in the first sentence of the specification. In particular, the amendment submitted 3/17/97 incorrectly indicates that the instant application is a continuation of Serial No. 08/272275.

The following is a quotation of the second paragraph of 35 U.S.C.

112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 28 and 30-37 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 28 is indefinite because it recites "said recombinant protein or portion thereof having the amino acid sequence represented by ... or ..." and it is not immediately clear what protein or portion thereof the sequences recited are intended to represent. From Applicant's remarks it is believed that the specific sequence from Fig. 1 is intended to be the recited "protein" and at least part of the specific sequence from Fig. 2 is intended to be the "portion thereof." If such is the case, it is suggested that the language be amended to

Art Unit: 1643

clarify, e.g., "wherein said recombinant protein has the sequence ... and said portion has the sequence ...", or other language that would provide clarification.

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 28 and 30-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Patent 5,106,726 to Wang, of record. Wang discloses that the HCV core or capsid protein, amino acids 1-120, has important immunodominant epitopes and shows immunoreactivity of core proteins. Wang teaches assaying sera for antibodies against HCV (NANBV) using solid phase coated with synthetic peptides that include amino acid residue sequences from the HCV core (see Wang, Example 14 and Table 7, especially peptides VIIIE, IXD, and IXE). With respect to claims 30-37, various immunoassay formats and labels would have been obvious over the immunoassays of Wang since Wang suggests that other formats may be used (see, e.g., col. 29, lines 32-42). The recited core protein would have

Art Unit: 1643

been obvious over Wang because any minor differences are no more than one would have expected between different HCV isolates. Wang does not exemplify producing recombinant HCV polypeptides, but suggests that recombinant antigens may be substituted for synthetic antigens, especially for making HCV peptides of more than about 50 amino acids: Wang, col. 25, lines 29-42. It would have been obvious to one of ordinary skill in the art to produce and use the HCV capsid of Wang recombinantly as suggested by Wang in order to gain the advantages of producing peptides by recombinant means, e.g., to obtain a stable, plentiful supply of peptides that are free of contamination with other HCV antigens. One of ordinary skill in the art at the time the invention was made would have recognized that there are advantages to using recombinant antigens and if one were willing to forego the advantages of synthetics in favor of the known advantages of recombinants, then one would have been motivated to select recombinant antigens for use in immunoassays.

Claim 28 is rejected under 35 U.S.C. § 102(e) as anticipated by or, in the alternative, under 35 U.S.C. § 103 as obvious over patent 5,350,671 to Houghton et al., of record. It is believed that Houghton anticipates an immunoassay using as recombinant antigen the sequence represented in Fig. 1, amino acid residues 1-120 (see, e.g., col. 81, lines 55-65), but if not, any minor differences in the antigen sequence 1-120 as taught by Houghton are no more than one would have expected to occur from one HCV isolate to another.

Claims 30-37 are rejected under 35 U.S.C. § 103 as obvious over the patent to Houghton et al. as cited and applied above. Houghton

Art Unit: 1643

teaches use of HCV core antigens as discussed above and additionally teaches different assay formats and labels. Thus it would have been obvious to use the HCV core antigen in Fig. 1, amino acid residues 1-120, as discussed above, with various formats and labels because Houghton suggests these as being useful with any HCV antigen.

Claim 28 and 30-37 are rejected under 35 U.S.C. § 103 as being unpatentable over US Patent US Patent 5,106,726 to Wang or over US Patent 5,350,671 to Houghton et al., both of record, each in view of US Patent 5,654,176 to Smith. An immunoassay using as antigen the sequence represented by Fig. 2, amino acid residues 1-315, which includes HCV core antigen sequence 1-74, would have been an obvious variant of either Wang's peptide VIIIE, which has HCV core antigen amino acids 2-62, or of Houghton's disclosed recombinant antigen CA279a which contains HCV core antigen 1-84 (see, e.g., col. 83, lines 35-55) because HCV 1-74 would have nearly 90% of the amino acids present in 1-84 and the first 74 amino acids are the same, allowing for minor variations which may occur from one viral isolate to another. Houghton teaches expression of HCV antigens as fusion proteins, but neither Wang nor Houghton teaches expression of known HCV core antigens as a recombinant fusion protein with glutathione transferase. Smith teaches a system for production of recombinant fusion proteins with glutathione transferase that is generally applicable for obtaining any desired protein fused to GST, as well as the advantages of GST fusion protein production, i.e., the fused polypeptides are generally soluble and can be readily purified from bacterial lysates without altering the antigenicity or destroying the functional activity of the desired protein (col. 1, lines 13-20). It

Art Unit: 1643

would have been obvious to one of ordinary skill in the art to make and use HCV core antigenic polypeptides as suggested by Wang or Houghton in the form of fusion proteins with GST as taught by Smith in order to obtain the disclosed advantages of GST fusion proteins of Smith, i.e., in order to have soluble, easily purified fusion proteins that retain the antigenicity of the desired polypeptide.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Wortman whose telephone number is (703) 308-1032. The examiner can normally be reached on Monday through Thursday from 8:00 am to 5:30 pm. The examiner can also be reached on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Marian Knode, can be reached on (703) 308-4311. The fax phone number for this Group is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.



Donna C. Wortman, Ph.D.
Patent Examiner

September 25, 1998

File

PTO 892		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		SERIAL NUMBER 08/819857		Art Unit 1643		Attachment to Paper Number 38	
NOTICE OF REFERENCES CITED									
				Applicant(s): Zebedee et al.					
U.S. PATENT DOCUMENTS									
*		DOCUMENT NUMBER	DATE	NAME(S)	CLASS	SUBCLASS	FILING DATE		
		5,654,176	8/1997	Smith	435	69.7	9/1994		
FOREIGN PATENT DOCUMENTS									
*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUBCLASS	PERTINENT DRW. SPEC	
OTHER REFERENCES (INCLUDING AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.)									
EXAMINER D. Wortman			DATE 9/21/98		* A COPY OF THIS REFERENCE IS NOT BEING FURNISHED WITH THIS OFFICE ACTION. (SEE MPEP SECTION 707.05(a). PAGE 1 OF 1				



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

(41)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/819,857	03/17/97	ZEBEDEE	2573763066

HM12/0604

EDWARD P. GAMSON
WELSH AND KATZ LTD
22ND FLOOR
120 SOUTH RIVERSIDE PLAZA
CHICAGO IL 60606

EXAMINER
WORTMAN, D.

ART. UNIT	PAPER NUMBER
1643	39

DATE MAILED:

06/04/99

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Notice of AbandonmentApplication No.
08/819,857Applicant(s)
Zebedee et al.Examiner
Donna C. Wortman, Ph.D.Group Art Unit
1643

This application is abandoned in view of:

- ☒ applicant's failure to timely file a proper response to the Office letter mailed on Sep 28, 1998.
- ☐ A response (with a Certificate of Mailing or Transmission of _____) was received on _____, which is after the expiration of the period for response (including a total extension of time of _____ month(s)) which expired on _____.
- ☐ A proposed response was received on _____, but it does not constitute a proper response to the final rejection.
(A proper response to a final rejection consists only of: a timely filed amendment which places the application in condition for allowance; a Notice of Appeal; or the filing of a continuing application under 37 CFR 1.62 (FWC)).
- ☒ No response has been received.
- ☐ applicant's failure to timely pay the required issue fee within the statutory period of three months from the mailing date of the Notice of Allowance.
- ☐ The issue fee (with a Certificate of Mailing or Transmission of _____) was received on _____.
- ☐ The submitted issue fee of \$ _____ is insufficient. The issue fee required by 37 CFR 1.18 is \$ _____.
- ☐ The issue fee has not been received.
- ☐ applicant's failure to timely file new formal drawings as required in the Notice of Allowability.
- ☐ Proposed new formal drawings (with a Certificate of Mailing or Transmission of _____) were received on _____.
- ☐ The proposed new formal drawings filed _____ are not acceptable.
- ☐ No proposed new formal drawings have been received.
- ☐ the express abandonment under 37 CFR 1.62(g) in favor of the FWC application filed on _____.
- ☐ the letter of express abandonment which is signed by the attorney or agent of record, the assignee of the entire interest, or all of the applicants.
- ☐ the letter of express abandonment which is signed by an attorney or agent (acting in a representative capacity under 37 CFR 1.34(a)) upon the filing of a continuing application.
- ☐ the decision by the Board of Patent Appeals and Interferences rendered on _____ and because the period for seeking court review of the decision has expired and there are no allowed claims.
- ☐ the reason(s) below:

DONNA WORTMAN
PRIMARY EXAMINER

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

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07/573 643

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08/819857
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I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. _____, page _____, line _____

United States Patent Number 6692751, column _____, line _____, or

WIPO Pub. No. _____, page _____, line _____

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- the pending application as originally filed.

Henry Duong
Signature

HENRY DUONG
Typed or printed name

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703 916 1500

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2-3-06

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573643

FILE 'USPAT' ENTERED AT 10:32:25 ON 30 MAR 92

* WELCOME TO THE
* U. S. PATENT TEXT FILE

=> file jpo

FILE 'JPOABS' ENTERED AT 10:32:49 ON 30 MAR 92

* JAPANESE PATENT ABSTRACTS
*
* CURRENTLY, DATA IS LOADED THROUGH THE ABSTRACT PUBLICATION
* DATE OF AUGUST 30, 1991.
* THE LATEST GROUPS RECEIVED ARE: C0862 E1105, M1150 & P1245.

=> s hepatitis

L1 293 HEPATITIS

=> s non (2w) hepatitis or (nanb?) or hcv

59371 NON

293 HEPATITIS

14 NON (2W) HEPATITIS

11 NANB?

3 HCV

L2 27 NON (2W) HEPATITIS OR (NANB?) OR HCV

=> s l2 and l1

L3 15 L2 AND L1

=> s 1-15 all

03-30676

Feb. 8, 1991

L3: 1 of 15

DNA OF NON-A, **NON**-B **HEPATITIS** VIRUS, THE CLONE AND ITS
PREPARATION

INVENTOR: MAKOTO HATTORI, et al. (4)

ASSIGNEE: SANWA KAGAKU KENKYUSHO CO LTD, et al. (40)

APPL NO: 01-163715

DATE FILED: Jun. 28, 1989

PATENT ABSTRACTS OF JAPAN

ABS GRP NO: C0825

ABS VOL NO: Vol. 15, No. 154

ABS PUB DATE: Apr. 18, 1991

INT-CL: C12N 15*51; //A61K 39*29

ABSTRACT:

NEW MATERIAL: A single stranded DNA containing about 850 nucleotides or a
duplex DNA comprising the single stranded DNA and complementary DNA,
having a basic sequence to code part amino acid sequence of gene of

non-A, **non**-B **hepatitis** virus.

USE: Producing a raw material for diagnosticum and medicine for non-A, **non**-B **hepatitis**.

PREPARATION: For example, RNA is extracted from particle fraction of plasma derived from patient of non-A, **non**-B **hepatitis** and purified. EcoRI linker is added to duplex DNA fragment prepared by using the purified RNA as a template. Then the resulting substance is digested with restriction enzyme EcoRI, the prepared duplex DNA fragment is optionally separated into a single stranded DNA to give DNA having a basic sequence to code part of amino acid sequence of non-A, **non**-B **hepatitis** virus gene.1

02-186990

Jul. 23, 1990

L3: 2 of 15

cDNA CLONE OF POST-TRANSFUSION NON-A **NON**-B **HEPATITIS** VIRUS
(**NANB**) AND USE THEREOF

INVENTOR: MAKOTO HATTORI, et al. (4)
ASSIGNEE: SANWA KAGAKU KENKYUSHO CO LTD, et al. (90)
APPL NO: 01-4059
DATE FILED: Jan. 10, 1989
PATENT ABSTRACTS OF JAPAN
ABS GRP NO: C0767
ABS VOL NO: Vol. 14, No. 459
ABS PUB DATE: Oct. 4, 1990
INT-CL: C12N 15*51; A61K 39*29; C12N 7*02

ABSTRACT:

NEW MATERIAL: A cDNA clone such as phage clone YS1 or phage clone YS2 containing a nucleotide of about 5.4Kb coding the amino acid sequence of a post-transfusion non-A **non**-B **hepatitis** virus (**NANB**) and prepared by adding an EcoRI linker to a duplex cDNA fragment prepared by using a template consisting of a refined RNA existing in a particle fraction separated from serum and substituting and inserting the addition product to an EcoRI site of a lambda gt10 vector.

USE: Agent for the diagnosis, prevention and remedy of non-A **non**-B **hepatitis**. Blood cleaning agent for transfusion.

PREPARATION: The objective cDNA clone can be prepared e.g. by adding 20% polyethylene glycol to a plasma originated from a non-A **non**-B **hepatitis** patient, centrifuging the mixture at a high speed, solubilizing the precipitate with TEN buffer solution, etc., centrifuging at a high speed to collect purified RNA, preparing a duplex cDNA using the RNA as a template, adding an EcoRI linker to the cDNA and substituting and inserting the addition product to the EcoRI site of a lambda gt10 vector.e

01-124387

May 17, 1989

L3: 3 of 15

MANIFESTATION VECTOR HAVING DNA CODING NON-A **NON**-B **HEPATITIS**
SPECIFIC ANTIGEN, TRANSFORMANT AND PRODUCTION OF SAID ANTIGEN

INVENTOR: TATSURO SHIBUI, et al. (6)
ASSIGNEE: MITSUBISHI KASEI CORP
APPL NO: 62-283990
DATE FILED: Nov. 10, 1987
PATENT ABSTRACTS OF JAPAN
ABS GRP NO: C626
ABS VOL NO: Vol. 13, No. 367
ABS PUB DATE: Aug. 15, 1989
INT-CL: C12N 15*00; A61K 39*29; C12N 1*20; C12P 21*02; //(C12 P21*02;
C12R 1:19)

ABSTRACT:

PURPOSE: To produce a non-A **non**B **hepatitis** specific antigen, by introducing a DNA-containing DNA fragment coding a non-A **non**B **hepatitis** specific antigen in a cloning site existing at the downstream side of a promoter, thereby forming a manifestation vector.

CONSTITUTION: A DNA fragment containing a DNA coding a non-A **non**B **hepatitis** specific antigen is introduced into a cloning site existing at the downstream side of a promoter of manifestation vector. The obtained manifestation vector containing the DNA fragment is introduced into a host to effect the transformation of the host and the resultant transformant is cultured. The non-A **non**B **hepatitis** specific antigen produced and accumulated in the cultured product is separated, therefrom a large amount of non-A **non**B **hepatitis** specific antigen protein can be produced by this process at a low cost.

01-90197

Apr. 6, 1989
PEPTIDE

L3: 4 of 15

INVENTOR: SHIGETADA NAKANISHI, et al. (5)
ASSIGNEE: MITSUBISHI KASEI CORP
APPL NO: 62-246952
DATE FILED: Sep. 30, 1987
PATENT ABSTRACTS OF JAPAN
ABS GRP NO: C616
ABS VOL NO: Vol. 13, No. 302
ABS PUB DATE: Jul. 12, 1989
INT-CL: C07K 7*10; G01N 33*576; //A61K 39*29; C12N 15*00; C12P 21*00;
C07K 99:00

ABSTRACT:

NEW MATERIAL: A peptide, specifically reactive with a non-A, **non**B type **hepatitis** antigen mouse monoclonal antibody and having an amino acid sequence expressed by the formula or partial sequence thereof.

USE: A diagnostic reagent and vaccine for non-A, **non**B type **hepatitis** for screening transfusion blood or blood pharmaceuticals.

PREPARATION: For example, analysis of hydrophilicity and prediction of secondary structure of antigenic protein are carried out on the basis of an amino acid sequence found from the base sequence of a gene capable of coding a non-A, **non**-B type **hepatitis** antigen to estimate a part which is a hydrophilic region for determining antigenicity on an antigenic protein from a part of a secondary structure, such as .alpha.-helix or turn. A peptide constituting the estimated part for determining the antigenicity is then synthesized by using an automatic synthetic apparatus for the peptide by a conventional method to afford the aimed peptide having the amino acid sequence expressed by the formula or partial sequence thereof.

64-2576

Jan. 6, 1989
DNA FRAGMENT

L3: 5 of 15

INVENTOR: KAZUNOBU TAKAHASHI, et al. (6)
ASSIGNEE: MITSUBISHI KASEI CORP.
APPL NO: 62-140586
DATE FILED: Jun. 4, 1987
PATENT ABSTRACTS OF JAPAN
ABS GRP NO: C588
ABS VOL NO: Vol. 13, No. 171
ABS PUB DATE: Apr. 24, 1989
INT-CL: C12N 15*00; // (C12 N15*00; C12R 1:91)

ABSTRACT:

PURPOSE: To provide a DNA fragment containing a base sequence coding a non-A, **non**-B **hepatitis** specific antigen protein and useful for the mass-production of a non-A, **non**-B **hepatitis** specific antigen protein by a recombinant DNA technique.

CONSTITUTION: A liver tissue of a human or chimpanzee affected with non-A, **non**-B **hepatitis** is homogenized in an aqueous solution of guanidium thiocyanate and whole RNA is separated as a precipitate by an equilibrium density gradient ultracentrifugation using cesium chloride. The separated whole RNA is purified by the extraction with phenol and the precipitation with ethanol. The RNA is further purified by oligo(dT)-cellulose column chromatography to separate a poly(A)-containing RNA, which is used as a raw material for mRNA. The objective DNA is determined from the mRNA through a cDNA library. The DNA is composed of a sequence of 1,333 bases.

61-176856

Aug. 8, 1986
NON-A **NON**-B TYPE **HEPATITIS** ANTIGEN

L3: 6 of 15

INVENTOR: ISAO ONO, et al. (4)
ASSIGNEE: MITSUBISHI CHEM IND LTD, et al. (4)
APPL NO: 60-18201
DATE FILED: Feb. 1, 1983
PATENT ABSTRACTS OF JAPAN
ABS GRP NO: P531
ABS VOL NO: Vol. 10, No. 389

ABS PUB DATE: Dec. 26, 1986
INT-CL: G01N 33*576; A61K 39*00; A61K 39*29; C12N 15*00; C12P 21*00;
G01N 33*577; //C07K 15*04

ABSTRACT:

PURPOSE: To make the diagnosis of a non-A **non**B **hepatitis** infection history, etc. possible by using an antigen which has about 1.17 approx. 1.26 density by a sucrose density-gradient centrifugation method and reacts with the antibody obtd. by transforming the lymphocyte of an individual body generated with the non-A **non**B type **hepatitis** by EB virus to obtain the positive culture cells for the non-A **non**B type **hepatitis** associated antibody then cloning the same.

CONSTITUTION: The liver tissue of the individual body generated with the non-A **non**B type **hepatitis** is homogenized and is then centrifugated for about 30 min. approx. 1 hr. at about 8,000 approx. 1,000 rpm, then the tissue is subjected to ultracentrifugation at about 100,000g, by which the precipitate is obtd. The precipitate is further subjected to the sucrose density-gradient centrifugation by cane sugar, CsCl, KBr, etc. by which the precipitate is refined. The refining is executed by using the following antibody while assaying the antigen: The antibody obtd. by transforming the lymphocyte of the individual body generated with the non-A **non**B **hepatitis** by the EB virus to obtain the positive culture cell for the non-A **non**B type **hepatitis** associated antibody, then cloning the same is used.

61-36196

Mar. 20, 1986

L3: 7 of 15

MONOCLONAL ANTIBODY

INVENTOR: ISAO ONO, et al. (2)
ASSIGNEE: ISAO ONO, et al. (4)
APPL NO: 59-147355
DATE FILED: Jul. 16, 1984
PATENT ABSTRACTS OF JAPAN
ABS GRP NO: C363
ABS VOL NO: Vol. 10, No. 219
ABS PUB DATE: Jul. 31, 1986
INT-CL: C07K 15*04; A61K 39*29; A61K 39*395; G01N 33*576; G01N 33*577;
//C12N 15*00; C12P 21*00

ABSTRACT:

PURPOSE: To provide the titled human and chimpanzee-type antibody reactive specifically with the antigen developed in hepatic cell in the crisis of a non-A non-B hepatitis of chimpanzee and man, and prepared by using the cloned cell of lymphocyte positive to the antibody relating to the chimpanzee and human non-A **non**B **hepatitis**.

CONSTITUTION: The peripheral blood lymphocyte of convalescent chimpanzee or man of non-A **non**B **hepatitis** is transformed by Epstein-Barr

virus to obtain culture cell positive to the antibody relating to non-A **non**B **hepatitis**, and the cell is cloned by a soft agar method, critical dilution method, etc. to obtain a cell strain (cloned strain) capable of producing the objective antibody. The obtained cloned strain is proliferated in e.g. a serum-free medium containing 0.5% bovine serum albumin, and the supernatant liquid is collected, subjected to ultrafiltration (to remove a fraction having a molecular weight of 100,000), and purified by gel-filtration chromatography (with 0.2M boric acid buffer solution of 9.0pH) to obtain the objective antibody.

61-25484

Feb. 4, 1986

L3: 8 of 15

CELL STRAIN PRODUCING ANTIBODY

INVENTOR: ISAO ONO, et al. (2)
ASSIGNEE: ISAO ONO, et al. (3)
APPL NO: 59-147354
DATE FILED: Jul. 16, 1984
PATENT ABSTRACTS OF JAPAN
ABS GRP NO: C355
ABS VOL NO: Vol. 10, No. 178
ABS PUB DATE: Jun. 21, 1986
INT-CL: C12N 5*00; //A61K 39*29; A61K 39*395; C07K 15*04; C12N 15*00;
C12P 21*00; G01N 33*376; G01N 33*377

ABSTRACT:

PURPOSE: To provide a cell strain originated from human and chimpanzee, capable of producing a monoclonal antibody reactive specifically to an antigen developing in the hepatic cell of non-A **non**B **hepatitis** and the screening of serum, etc.

CONSTITUTION: The peripheral blood lymphocyte of chimpanzee or human of the coalescence of non-A **non**B **hepatitis** is transformed with Epstein-Barr virus (EB virus), and the obtained culture cell positive to the antibody relating to non-A **non**B **hepatitis** is cloned to obtain the cell strain capable of producing the objective antibody. For example, the peripheral blood of a chimpanzee or human of the coalescence of non-A **non**B **hepatitis** is collected and added with heparin, and lymphocyte is separated from the blood by centrifugal separation. Separately, the cell producing and releasing EB virus is cultured in a medium, and the supernatant liquid of the culture product is separated to obtain a virus source. The virus source is made to contact with the above lymphocyte, inoculated in a micro-titer plate for tissue culture at various cultivation densities, and cultured to obtain the objective strain.

60-176600

Sep. 10, 1985

L3: 9 of 15

METHOD FOR MEASURING ACTIVITY OF GUANASE

INVENTOR: NOBUYUKI IWAMOTO, et al. (2)
ASSIGNEE: KK FUJIMOTO RINSHIYOU KENSA KENKYUSHO
APPL NO: 59-34760

DATE FILED: Feb. 24, 1984
PATENT ABSTRACTS OF JAPAN
ABS GRP NO: C325
ABS VOL NO: Vol. 10, No. 23
ABS PUB DATE: Jan. 29, 1986
INT-CL: C12Q 1*48; G01N 33*50

ABSTRACT:

PURPOSE: To determine accurately, rapidly and easily the activity of guanase useful for non-A or **non** B **hepatitis**, by using a measuring reagent containing guanaine, xanthine oxidase, etc.

CONSTITUTION: A buffer solution of 6. approx. 9pH containing guanine or oxidized form tetrazolium or a halide thereof and xanthine oxidase is used as a measuring reagent, and a humoral sample is added thereto to convert guanine into xanthine by guanase in the sample. The resultant xanthine is converted into uric acid by xanthine oxidase, and the oxidized form tetrazolium is reduced to give reduced form tetrazolium (formazan) by superoxide anion. The absorbance of a characteristic absorption band of the formed formazan is measured.

60-09430

May 20, 1985

L3: 10 of 15

NON-A.multidot.**NON**-B-TYPE **HEPATITIS** RELATING ANTIGEN

INVENTOR: HITOSHI OOHORI, et al. (1)
ASSIGNEE: SENDAI BISEIBUTSU KENKYUSHO
APPL NO: 58-197356
DATE FILED: Oct. 20, 1983
PATENT ABSTRACTS OF JAPAN
ABS GRP NO: C303
ABS VOL NO: Vol. 9, No. 226
ABS PUB DATE: Sep. 13, 1983
INT-CL: A61K 39*00; //A61K 35*22; G01N 33*576

ABSTRACT:

PURPOSE: To provide the titled antigen composed of the protein recovered from the urine, blood, etc. of a non-A.multidot.**non**-B-type **hepatitis** patient, and useful for the remedy and diagnosis of non-A.multidot.**non**-B-type **hepatitis**.

CONSTITUTION: The urine, blood (preferably blood plasma), etc. of a non-A.multidot.**non**-B-type **hepatitis** patient is used as a raw material, is subjected to a proper combination of the concentration, the fractionation taking advantage of the solubility difference, the ion exchange material treatment, the gel filtration, etc. to obtain the objective SO antigen having the following characteristics: (i) electrophoretic analysis, moves toward the .beta.-region; (ii) molecular weight, about 250,000, decomposed to a fraction having a molecular weight of about 38,000 by electrophoresis; (iii) electron microscopic observation, granular structure having a diameter of 1nm; (iv)

exhibiting the antigenicity to animals; and (v) causing strong agglutination reaction with the serum and urine of only the non-A, multidot. ****non**B-type hepatitis** patient by the RPHA reagent prepared from the refined antigen. A reagent to detect the relating antibody can be produced by bonding said antigen with a carrier, and a vaccine is prepared by treating the antigen at 60 degrees C. for 10hr or treating with formalin.

58-183629

Oct. 26, 1983

L3: 11 of 15

MONOCLONAL ANTIBODY AND DIAGNOSTIC AGENT RELATING TO NON-A AND ****NON**B** TYPE ****HEPATITIS****

INVENTOR: TOSHITAKA AKATSUKA, et al. (2)

ASSIGNEE: EISAI KK

APPL NO: 57-65430

DATE FILED: Apr. 21, 1982

PATENT ABSTRACTS OF JAPAN

ABS GRP NO: C206

ABS VOL NO: Vol. 8, No. 15

ABS PUB DATE: Jan. 21, 1984

INT-CL: A61K 39*395; A61K 39*44; G01N 33*54

ABSTRACT:

PURPOSE: The titled monoclonal antibody that is obtained by isolating from autaptic livers with non-A and ****non**B type hepatitis** and purifying the product, thus being used as an ingredient of a diagnostic agent for non-A and non-B hepatitis, because of its showing characteristic antigen-antibody reaction with antigens relating to non-A and ****non**B type hepatitis**.

CONSTITUTION: The objective monoclonal antibody is obtained by isolating from autaptic livers with non-A and ****non**B type hepatitis** and purifying the product, shows a characteristic reaction with antigens relating to the above ****hepatitis**** and has following physical and chemical properties: molecular weight, more than 1,500,000 (by the gel filtration method); sedimentation constant (10. sup.-. sup.1. sup.3), 51.5S (by the ultracentrifugation method); floating density (g/cm. sup.3), 1.15, approx. 1.25 (in CsCl or KBr); particle size (nm), 26, approx. 37; electric mobility, in the .alpha.-sub.2-.alpha.-sub.1 globulin region (in the agarose gel). It is used as a diagnostic agent containing the antibody as a major ingredient, e.g., in the reverse passive hemagglutination method using sheep sensitized erythrocytes or the antibody sandwich method using sensitized glass beads.

56-753

Jan. 5, 1983

L3: 12 of 15

NON-A AND ****NON**B** TYPE ****HEPATITIS**** RELATED ANTIBODY AND DETECTION REAGENT

INVENTOR: JIYUNICHI FUJIMATSU, et al. (2)

ASSIGNEE: EISAI KK

APPL NO: 56-97425

DATE FILED: Jun. 25, 1981

PATENT ABSTRACTS OF JAPAN

ABS GRP NO: P185

ABS VOL NO: Vol. 7, No. 70

ABS PUB DATE: Mar. 23, 1983

INT-CL: G01N 33*54; A61K 39*395

ABSTRACT:

PURPOSE: To obtain a superior detection reagent of non-A and **non**-B type **hepatitis**, by combining antigen having a specific property which is separated and refined from non-A and **non**-B type **hepatitis** part liver and a singular antibody obtained by injecting to an animal with a minute particle, enzyme etc.

CONSTITUTION: Non-A and **non**-B **hepatitis** antigen having .gtoreq.150 ten thousand molecular weight by measured value of a gel filtration method, 51.5.times.10.^{sup.1}.sup.39 precipitation constant by an ultra centrifugal analysing method, 1.15.approx.1.25g/cm.^{sup.3} floating density in CsCl and KBr, 26.approx.37nm grain diameter and .alpha..sub.2-.alpha..sub.1 electric transfer degree in globulin domain (in agarose gel), is obtained by separating from non-A and **non**-B **hepatitis** part liver and refining it. Immunity is given to a house rabbit by this antigen and an antibody is obtained by carrying out IgG refining graduation of an antiserum. The highly accurate detection is made possible by using a combined body combined this antibody with fine particle of a red blood corpuscle of sheep etc., an isotope of .sup.1.sup.2.sup.51 etc., or an enzyme of alkali phosphatase etc. for the detection of non-A and non-B hepatitis antigen or using for a reagent for diagnosis of a patient or inspection of blood for blood transfusion.

57-198867

Dec. 6, 1982

L3: 13 of 15

NON-A, **NON**-B **HEPATITIS** RELATED ANTIGEN AND DIAGNOSTIC THEREFOR

INVENTOR: JIYUNICHI FUJIMATSU, et al. (2)

ASSIGNEE: EISAI KK

APPL NO: 56-63736

DATE FILED: Jun. 2, 1981

PATENT ABSTRACTS OF JAPAN

ABS GRP NO: P179

ABS VOL NO: Vol. 7, No. 51

ABS PUB DATE: Feb. 26, 1983

INT-CL: G01N 33*54; A61K 39*29

ABSTRACT:

PURPOSE: To enable a reliable diagnosis and curing of non-A, **non**-B **hepatitis**, by using a non-A, **non**-B **hepatitis** related antigen separated and refined from an autopsy liver of a non-A, **non**-B **hepatitis** patient, or a conjugate of said antigen with a sheep erythrocyte, an isotope, an enzyme or the like as a diagnostic.

CONSTITUTION: A non-A, **non**-B **hepatitis** related antigen, which has

MW of 1,500,000 or more (by a gel filtration method), a sedimentation constant (10^{sup.} - 1^{sup.} - 2^{sup.}) of 51.5S (by an ultracentrifugal analysis), a buoyant density (g/cm^{sup.} 2) of 1.15 approx. 1.25 (in cesium chloride and in KBr), a particle diameter (nm) of 26 approx. 37, and an electrophoretic mobility in an .alpha..sub.2-.alpha..sub.1 globulin region in agarose gel, is separated and refined from an autopsy liver of a non-A, **non-B hepatitis** patient by a specified treatment. A conjugate of the obtained antigen with a minute particle such as sheep erythrocyte, an isotope such as ^{sup.}1^{sup.} - ^{sup.}2^{sup.} - ^{sup.}51, alkali phosphatase or the like is prepared to obtain a diagnostic for immunological analysis. Thus a diagnostic which clearly distinguishes non-A, **non-B hepatitis** from other **hepatitis**.

57-175127

Oct. 28, 1982

L3: 14 of 15

SUBSTANCE AND VACCINE RELATED TO **HEPATITIS** **NANB**-1 AND **NANB**-2 VIRAL ANTIGEN

INVENTOR: KOUJI YOSHIKAWA, et al. (1)

ASSIGNEE: TETSUO NAKAMURA

APPL NO: 56-50221

DATE FILED: Apr. 21, 1981

PATENT ABSTRACTS OF JAPAN

ABS GRP NO: C147

ABS VOL NO: Vol. 7, No. 19

ABS PUB DATE: Jan. 25, 1983

INT-CL: A61K 39*29

ABSTRACT:

PURPOSE: To obtain the titled vaccine, having a high immunogenicity without infection, and useful for a detecting reagent of the titled viral antigen, by treating **hepatitis** **NANB**-1 and **NANB**-2 viral particles with an organic solvent, and heat-treating the particles.

CONSTITUTION: Viral particles, obtained from the blood serum of the **hepatitis** **NANB**-1 in the acute stadium, and found to be capable of agglutinating with the **hepatitis** **NANB**-1 blood serum in the decubation and infecting and developing the typical **hepatitis** **NANB**-1 in sensitive animals are inactivated by the addition of an organic solvent, e.g. 37% formalin, and the heat-treatment (50 degrees C. for 10hr) to give a **hepatitis** **NANB**-1 viral vaccine. Similarly, the **hepatitis** **NANB**-2 viral vaccine is obtained. The resultant respective specific antibodies of the hepatitic viruses permit the detection of the new **hepatitis** **NANB**-1 and **NANB**-2 viral antigens capable of infecting and developing the **hepatitis** non-A and non-B.

55-122156

Sep. 19, 1980

L3: 15 of 15

C-TYPE **HEPATITIS** VIRUS-ASSOCIATED ANTIGEN

INVENTOR: RIYOUICHI SHIROJI, et al. (1)

ASSIGNEE: KAGAKU OYOBI KETSUSEIRIYOHOU KENKYUSHO

APPL NO: 54-30332

DATE FILED: Mar. 14, 1979
PATENT ABSTRACTS OF JAPAN
ABS GRP NO: P040
ABS VOL NO: Vol. 4, No. 180
ABS PUB DATE: Dec. 12, 1980
INT-CL: G01N 33*54; A61K 39*29

ABSTRACT:

PURPOSE: To obtain C-type **hepatitis** virus-associated antigen by separating a special antigen from blood plasma (serum) of a patient diagnosed as non-A-type or **non**-B-type **hepatitis** after he had been subjected to transfusion of HBs antigen-negative blood.

CONSTITUTION: Blood plasma (serum) of a patient diagnosed as non-A-type or **non**-B-type **hepatitis** with multi-peak rise in GPT in particular and with a comparatively long period of incubation, obtained in the acute stage is used as a starting substance. This substance is subjected to gel filtration to obtain fractions (based on absorbance measurement of about 280nm). Among these fractions, on corresponding to a third peak P.sub.3 (refer to the drawing) is collected and subjected to column chromatography using an ion exchanger. The resulting product is condensed as necessary by using a precipitation method with polyethylene glycol, and refined by an ultra-centrifugal separation to obtain an objective antigen.

=> file uspat

FILE 'USPAT' ENTERED AT 10:36:30 ON 30 MAR 92

* WELCOME TO THE *
* U. S. PATENT TEXT FILE *

=> 436820/cclr

L4 119 436820/CCLR

=> d 1-10

1. 5,091,300, Feb. 25, 1992, Radio-immuno assay for hepatitis B virus PreS2 antibodies; William M. Hurri, et al., 435/5, 235-1, 910, 948; 436/501, 518, 534, 543, 547, 804, 808, **820** [IMAGE AVAILABLE]

2. 5,073,481, Dec. 17, 1991, Assay to detect the presence of live virus in vitro; Jerome B. Zeldis, et al., 435/5, 424/89; 435/29, 236; **436/820** [IMAGE AVAILABLE]

3. 5,061,619, Oct. 29, 1991, Immunoassay using antibody-antigen conjugates; Strathearn Wilson, et al., 435/5, 7.1, 7.9, 7.92, 7.94; 436/507, 509, 512, 513, 518, 536, 540, **820** [IMAGE AVAILABLE]

4. 5,030,535, Jul. 9, 1991, Membrane-strip reagent serodiagnostic apparatus and method; Roger M. Clemmons, 435/5, 422/56, 57, 58; 435/7.94,

208, 299, 300, 301, 311, 805, 810; 436/177, 178, 510, 528, 530, 531, 535, 608, 610, 611, 813, **820** [IMAGE AVAILABLE]

5. 4,932,494, Aug. 28, 1990, Assay to detect the presence of live non-A, non-B hepatitis agents in vitro; Jerome B. Zeldis, et al., 435/5, 29, 32, 236; **436/820** [IMAGE AVAILABLE]

6. 4,912,030, Mar. 27, 1990, Viral isolates and their use in diagnosis; Robin Weiss, et al., 435/5; 424/89, 937; 435/93, 188, 810, 974, 975; 436/518, 531, 543, 547, 804, 808, 809, 810, 815, **820**, 823

7. 4,879,219, Nov. 7, 1989, Immunoassay utilizing monoclonal high affinity IgM antibodies; Jack R. Wands, et al., 435/5; 424/1.1, 65.8, 69; 435/240.27, 948; 436/503, 504, 536, 537, 538, 539, 540, 541, 542, 804, 811, **820**, 530/387, 389, 826 [IMAGE AVAILABLE]

8. 4,871,659, Oct. 3, 1989, Reagent for detecting non-A, non-B viral hepatitis (NANBH) and an immunoenzymatic method for detecting NANBH antigens in fecal extracts; Jacques Pillot, 435/5; 422/61; 435/7.94, 810; 436/513, 531, 808, **820**

9. 4,853,326, Aug. 1, 1989, Carbohydrate perturbations of viruses or viral antigens and utilization for diagnostic prophylactic and/or therapeutic applications; Gerard A. Quash, et al., 435/5, 974; 436/507, 518, 543, 548, 812, **820**

10. 4,839,298, Jun. 13, 1989, Virus inactivating diluents used in immunoassays; John W. D. Kay, et al., 436/175; 424/89, 531; 435/5, 230, 974; 436/22, 174, 176, 536, **820**

=> d 11-20

11. 4,839,277, Jun. 13, 1989, Method for purification of HBc antigen and method for measurement of HBc antibody by using said purified HBc antigen; Keishin Sugahara, et al., 435/5, 69.3, 239; **436/820**; 935/68, 69

12. 4,837,167, Jun. 6, 1989, Immunoassay for multi-determinant antigens using high-affinity; Hubert J. A. Schoemaker, et al., 435/5; 424/86; 435/7.94; 436/513, 518, 536, 540, 542, 548, 804, 819, **820**, 530/387, 806; 935/107, 108, 110

13. 4,818,688, Apr. 4, 1989, Assays for antibody to hepatitis B core antigen; Marina Adamich, et al., 435/5; 424/85.8, 86; 435/7.93, 70.21, 172.2, 240.27, 810, 948; 436/518, **820**

14. 4,803,156, Feb. 7, 1989, Peptide-beta-lactamase conjugates for enzyme-linked immunoassays; Alexander R. Neurath, et al., 435/5, 7.92, 16, 19; **436/820**, 820; 930/142, 200, 221, 222, 223, 260, 310, DIG.820

15. 4,788,138, Nov. 29, 1988, Method to achieve a linear standard curve in a sandwich immunoassay; Ken-Kong Tung, et al., 435/5, 7.23, 7.4, 7.5, 7.7, 7.94; 436/513, 523, 534, 801, 803, 817; **820**, 827

16. 4,752,562, Jun. 21, 1988, Detection of serum antibody and surface antigen by radial partition immunoassay; Mark I. Shelman, et al., 435/5, 7.22, 7.31, 29; 436/514, 515, 519, 527, 535, 541, **020**

17. RE 32,696, Jun. 14, 1988, Enzymatic immunological method for determination of antigens and antibodies; Antonius H. W. M. Schuur, et al., 435/5, 7.93, 610; 436/510, 531, 532, 800, **020**

18. 4,727,019, Feb. 23, 1988, Method and apparatus for immunoassays; Gunars E. Valkirs, et al., 435/5, 6, 7.34, 7.4, 7.5, 287, 974; 436/513, 518, 527, 531, 548, 007, 010, **020**, 624

19. 4,707,542, Nov. 17, 1987, Immunogenic HbsAg derived from transformed yeast; Arthur Friedman, et al., 530/371; 210/190.2, 502.1, 625; 424/89; 435/5, 69-3, 71.1, 172.3, 235.1, 239, 255, 803; **436/820**, 530/395, 415, 417, 806, 826

20. 4,707,439, Nov. 17, 1987, Screening test for reverse-transcriptase containing virus such as non-A, non-B hepatitis, NANBH; Belinda P. Seto, et al., 435/5; 424/3; 425/4; 6; **436/820**, 935/76 [IMAGE AVAILABLE]

=> d his

(FILE 'USPAT' ENTERED AT 10:32:25 ON 30 MAR 92)

FILE 'JPOABS' ENTERED AT 10:32:49 ON 30 MAR 92

L1 293 S HEPATITIS
L2 27 S NON (2W) HEPATITIS OR (NANB?) OR HCV
L3 15 S L2 AND L1

FILE 'USPAT' ENTERED AT 10:36:30 ON 30 MAR 92

L4 119 S 436820/CCLR

=> = 12

536033 NON
2749 HEPATITIS
100 NON (2W) HEPATITIS
200 NANB?
40 HCV
L5 321 NON (2W) HEPATITIS OR (NANB?) OR HCV

=> = 11

L6 2749 HEPATITIS

=> = 11 and 15

L7 2749 HEPATITIS
113 L1 AND L5

=> d 1-20

1. 5,099,002, Mar. 24, 1992, Sequential improved method for treatment of human blood-clotting factor products; Alan I. Rubinstein, 530/391; 424/530; 514/8, 12, 21; 530/380, 382, 383, 384 [IMAGE AVAILABLE]

2. 5,097,018, Mar. 17, 1992, Sequential heat treatment of blood-clotting factor products; Alan I. Rubinstein, 530/383; 424/530; 514/6, 12, 21; 530/381 [IMAGE AVAILABLE]

3. 5,094,960, Mar. 10, 1992, Removal of process chemicals from labile biological mixtures by hydrophobic interaction chromatography; Richard J. Bonomo, 436/178; 210/656; 435/69.5 [IMAGE AVAILABLE]

4. 5,087,572, Feb. 11, 1992, DNA encoding human plasminogen modified at the cleavage site; Francis J. Castellino, et al., 435/240.2, 217, 252.3, 255, 320.1; 536/87 [IMAGE AVAILABLE]

5. 5,077,193, Dec. 31, 1991, Non-A, **Non**-B **hepatitis** virus genome RNA, cDNA and virus antigen protein; Shunji Mishiro, et al., 435/5, 6; 436/94, 501; 536/26, 27, 28 [IMAGE AVAILABLE]

6. 5,077,192, Dec. 31, 1991, Method of detecting antigenic, nucleic acid-containing macromolecular entities; Tsanyang Liang, et al., 435/5, 6, 7.1, 7.2 [IMAGE AVAILABLE]

7. 5,075,425, Dec. 24, 1991, Process for the preparation of a pharmaceutical which contains IgG, IgA and IgM and can be administered intravenously; Ronald Kotitschke, et al., 530/387; 424/85.8 [IMAGE AVAILABLE]

8. 5,063,054, Nov. 5, 1991, Microbial products used for treatment of **hepatitis**; Joseph Chang, 424/92, 195.1, 520; 435/824 [IMAGE AVAILABLE]

9. 5,061,237, Oct. 29, 1991, Method of purifying whole blood; Reiner Gessler, et al., 604/5; 436/512 [IMAGE AVAILABLE]

10. 5,055,485, Oct. 3, 1991, Inactivation of viruses in cells and protein-containing compositions using aryl diol epoxides; Nicholas E. Geacintov, et al., 514/449; 424/529, 530, 531, 583; 435/1, 2; 514/2 [IMAGE AVAILABLE]

11. 5,041,078, Aug. 20, 1991, Photodynamic viral deactivation with sapphyrins; J. Lester Matthews, et al., 604/4; 540/145 [IMAGE AVAILABLE]

12. 5,036,072, Jul. 30, 1991, Antiviral agent; Tsunetaka Nakajima, et al., 514/274, 346, 351 [IMAGE AVAILABLE]

13. 5,032,511, Jul. 16, 1991, DNA fragments coding for antigens specific to non-A **non**-B **hepatitis**, expression vectors containing said DNA fragments, transformants and process for producing said antigens; Kazuhiro Takahashi, et al., 435/69.1, 91, 172.3, 233.1, 240.1, 252.31, 252.33, 320.1; 530/350; 536/27; 935/18, 27, 31, 41, 56, 57, 65, 70, 73, 74, 81 [IMAGE AVAILABLE]

14. 5,013,305, May 7, 1991, Needle safety system and method; Eric A. Opitz, et al., 604/192, 196, 263 [IMAGE AVAILABLE]

15. 5,005,793, Apr. 9, 1991, Pole clip needle cap holder; Richard A.

Shillington, 248/229, 230, 912 [IMAGE AVAILABLE]

16. 5,004,688, Apr. 2, 1991, Purification of **hepatitis** proteins; William S. Craig, et al., 435/69.3, 235.1; 530/350 [IMAGE AVAILABLE]

17. 4,994,433, Feb. 19, 1991, Heat treatment of lyophilized plasma fractions; Alan Rubinstein, 514/2; 424/530 [IMAGE AVAILABLE]

18. 4,994,046, Feb. 19, 1991, Needle guard for syringe; Vann T. Wesson, et al., 604/198; 128/919; 604/263 [IMAGE AVAILABLE]

19. 4,979,616, Dec. 25, 1990, Syringe disposal container; Dennis L. Clenton, 206/364, 523; 220/4.24 [IMAGE AVAILABLE]

20. 4,971,760, Nov. 20, 1990, Novel method for disinfecting red blood cells, blood platelets, blood plasma, and optical corneas and sclerae; Alan I. Rubinstein, 422/37, 28; 435/3; 514/833; 530/385 [IMAGE AVAILABLE]

=> s hepatitis/ti,ab

153 HEPATITIS/TI

235 HEPATITIS/AB

L3 253 HEPATITIS/TI,AB

=> s 18 and 17

L9 33 L8 AND L7

=> d 1-33

1. 5,099,002, Mar. 24, 1992, Sequential improved method for treatment of human blood-clotting factor products; Alan I. Rubinstein, 530/381; 424/530; 514/8, 12, 21; 530/380, 382, 383, 384 [IMAGE AVAILABLE]

2. 5,097,018, Mar. 17, 1992, Sequential heat treatment of blood-clotting factor products; Alan I. Rubinstein, 530/383; 424/530; 514/8, 12, 21; 530/381 [IMAGE AVAILABLE]

3. 5,077,193, Dec. 31, 1991, Non-A, **Non**-B, **hepatitis** virus genome RNA, cDNA and virus antigen protein; Shunji Mishiro, et al., 435/3, 6; 436/94, 501; 536/26, 27, 28 [IMAGE AVAILABLE]

4. 5,063,054, Nov. 5, 1991, Microbial products used for treatment of **hepatitis**; Joseph Chang, 424/92, 193.1, 520; 435/824 [IMAGE AVAILABLE]

5. 5,032,511, Jul. 16, 1991, DNA fragments coding for antigens specific to non-A **non**-B **hepatitis**, expression vectors containing said DNA fragments, transformants and process for producing said antigens; Kazuhiro Takahashi, et al., 435/69.1, 91, 172.3, 235.1, 240.1, 252.31, 252.32, 320.1; 530/350; 536/27; 935/18, 27, 31, 41, 56, 57, 65, 70, 73, 74, 91 [IMAGE AVAILABLE]

6. 5,004,688, Apr. 2, 1991, Purification of **hepatitis** proteins; William S. Craig, et al., 435/69.3, 235.1; 530/350 [IMAGE AVAILABLE]

7. 4,952,494, Aug. 28, 1990, Assay to detect the presence of live non-A, ~~non-B~~ hepatitis agents in vitro; Jerome B. Zeldis, et al., 433/5, 29, 32, 236; 436/820 [IMAGE AVAILABLE]

8. 4,871,659, Oct. 3, 1989, Reagent for detecting non-A, ~~non-B~~ viral hepatitis (~~NANBH~~) and an immunoenzymatic method for detecting ~~NANBH~~ antigens in fecal extracts; Jacques Pillot, 435/5; 422/61; 435/7.94, 810; 436/513, 531, 808, 820

9. 4,370,026, Sep. 26, 1989, Non-A, ~~non-B~~ hepatitis virus, methods of identification purification, characterization, diagnosis and immunization; Jack Wands, et al., 436/548; 424/85.8, 89

10. 4,820,805, Apr. 11, 1989, Undenatured virus-free trialkyl phosphate treated biologically active protein derivatives; Alexander R. Neurath, et al., 530/410; 424/89; 530/391, 406, 808, 829

11. 4,777,245, Oct. 11, 1988, Non-human primate monoclonal antibodies and methods; Steven K. H. Fong, et al., 530/387; 424/1.1, 86; 435/5, 7.23, 70.21, 172.2, 172.3, 180, 240.27, 948; 935/96 [IMAGE AVAILABLE]

12. 4,764,369, Aug. 16, 1988, Undenatured virus-free biologically active protein derivatives; Alexander R. Neurath, et al., 424/89, 85.8; 435/236; 514/8

13. 4,707,439, Nov. 17, 1987, Screening test for reverse-transcriptase containing virus such as non-A, ~~non-B~~ hepatitis, ~~NANBH~~; Belinda P. Seto, et al., 435/5; 424/3; 435/4, 6; 436/820; 935/76 [IMAGE AVAILABLE]

14. 4,702,909, Oct. 27, 1987, Non-A, ~~non-B~~ hepatitis antigen, antigen compositions, vaccine and diagnostic reagent; Victor M. Villarejos, et al., 424/89; 435/5, 235.1, 239; 436/5, 543, 820

15. 4,673,634, Jun. 16, 1987, Purified antigen from non-A, ~~non-B~~ hepatitis causing factor; Belinda Seto, et al., 435/5; 424/86, 89; 435/7.9, 810, 961; 436/543, 547, 820; 530/387, 395, 826

16. 4,615,886, Oct. 7, 1986, Utilizing a halohydrocarbon containing dissolved water to inactivate a lipid virus; Robert H. Purcell, et al., 514/2; 424/529, 530; 514/8

17. 4,591,505, May 27, 1986, Process for inactivating hepatitis B virus; Alfred M. Prince, 424/530; 435/236

18. 4,581,231, Apr. 8, 1986, Inactivation of viruses containing essential lipids; Robert H. Purcell, et al., 424/530; 435/238; 514/2; 530/383

19. 4,542,016, Sep. 17, 1985, Non-a ~~non-b~~ hepatitis surface antigen useful for the preparation of vaccines and methods of use; Christian Trepo, 424/86, 89

20. 4,540,573, Sep. 10, 1985, Undenatured virus-free biologically active

protein derivatives; Alexander R. Neurath, et al., 530/381; 424/529, 531, 534; 514/2. 6; 530/351, 359, 364, 360, 382, 363, 384, 385, 386, 387, 382, 393, 394, 389, 830, 831

21. 4,511,556, Apr. 16, 1985, Inactivation of a lipid virus; Robert H. Purcell, et al., 514/743; 424/89; 435/238; 514/758

22. 4,495,278, Jan. 22, 1985, Process for making novel blood clotting enzyme compositions; William R. Thomas, 435/5

23. 4,491,632, Jan. 1, 1985, Process for producing antibodies to **hepatitis** virus and cell lines therefor; Jack R. Wands, et al., 435/240.27; 424/86; 435/172.2; 935/103

24. 4,481,189, Nov. 6, 1984, Process for preparing sterilized plasma and plasma derivatives; Alfred M. Prince, 424/530; 514/2; 530/383

25. 4,464,474, Aug. 7, 1984, Non-A, **non**B **hepatitis** assay and vaccine; Pierre L. J. Coursaget, et al., 436/513, 531, 804, 820

26. 4,456,590, Jun. 26, 1984, Heat treatment of lyophilized blood clotting factor VIII concentrate; Alan Rubinstein, 530/383; 514/2

27. 4,438,098, Mar. 20, 1984, Heat treatment of a non-A, **non**B **hepatitis** agent to prepare a vaccine; Edward Tabor, et al., 424/89; 435/235.1, 236, 239

28. 4,395,395, Jul. 26, 1983, Detection of non-A, **non**B **hepatitis** associated antigen; Edward Tabor, et al., 424/89; 436/516; 530/306

29. 4,356,164, Oct. 26, 1982, Detection of non-A, **non**B **hepatitis** associated antigen; Edward Tabor, et al., 435/5, 7.25, 966; 436/515, 516, 520, 522, 533, 542, 820 [IMAGE AVAILABLE]

30. 4,314,997, Feb. 9, 1982, Purification of plasma protein products; Edward Shanbrom, 514/2, 8, 21

31. 4,291,020, Sep. 22, 1981, Inactivation of non-A, **non**B **hepatitis** agent; Edward Tabor, et al., 424/89; 435/238

32. 4,271,145, Jun. 2, 1981, Process for producing antibodies to **hepatitis** virus and cell lines therefor; Jack R. Wands, et al., 530/387; 424/86, 88, 89; 435/172.2, 240.27, 948; 935/70, 107, 108, 110

33. 4,021,540, May 3, 1977, Preparation of a **hepatitis** B immune globulin and use thereof as a prophylactic material; William Pollack, et al., 424/86; 436/544, 545, 804, 820; 530/387, 830, 831

=> d his

(FILE 'USPAT' ENTERED AT 10:32:25 ON 30 MAR 92)

FILE 'JPDBS' ENTERED AT 10:32:43 ON 30 MAR 92

L1 293 S HEPATITIS
L2 27 S NON (2W) HEPATITIS OR (NANB?) OR HCV
L3 15 S L2 AND L1

FILE 'USPAT' ENTERED AT 10:36:30 ON 30 MAR 92

L4 119 S 436820/CCLR
L5 321 S L2
L6 2749 S L1
L7 113 S L1 AND L5
L8 253 S HEPATITIS/TI, AB
L9 33 S L8 AND L7

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U.S. Patent & Trademark Office LOGOFF AT 10:41:29 ON 30 MAR 92

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FastDB - Fast Pairwise Comparison of Sequences
 Release 3.4

Results file wortman-616-fig1-n-geneseq.res made by maryh on Wed 25 Mar 92
 11058:10-PST.

Query sequence being compared: WORTMAN-616-FIG1 (1-240)
 Number of sequences searched: 12039
 Number of scores above cutoff: 3758

Results of the initial comparison of WORTMAN-616-FIG1 (1-240) with:
 Data bank : N-GeneSeq 5, all entries

10000-
 N
 U 5000-
 M --*
 B25
 E
 R
 O --*
 F30000- ** **
 S
 E 500- *
 Q *
 U35
 E
 N
 C
 E
 S40100- **
 50- *
 45 *
 50 10- *
 5- *

SCORE 0 1 26 1 52 1 78 1 104 1 130 156 182 208 234
 STDEV 0 1 2 3 4 5 6 7 8 9

PARAMETERS

Similarity matrix Unitary K-tuple 4
 Mismatch penalty 1 Joining penalty 30
 Gap penalty 1.00 Window size 32
 Gap size penalty 0.33
 Cutoff score 14
 Randomization group 0
 Initial scores to save 20 Alignments to save 10
 Optimized scores to save 20 Display context 10

SEARCH STATISTICS

Scores: Mean 17 Median 16 Standard Deviation 11.78

Times: CPU 00:02:00.02 Total Elapsed 00:04:07.00

Number of residues: 8958641
 Number of sequences searched: 12039
 Number of scores above cutoff: 3758

Cut-off raised to 19.
 Cut-off raised to 23.

The scores below are sorted by initial score.
 Significance is calculated based on initial score.

A 100% identical sequence to the query sequence was not found.

The list of best scores is:

Sequence Name	Description	Length	Init. Score	Opt. Score	Sig. Fra
**** 18 standard deviations above mean ****					
1. Q10566	Hepatitis C virus strain 1 DN	9185	234	234	18.42
2. Q05956	Sense strand of the compiled	9185	234	234	18.42
**** 17 standard deviations above mean ****					
3. Q11075	Fragment of hepatitis C virus	552	220	223	17.23

4. Q12242 Encodes PT-NANBH viral struct 2116 220 220 17.23

**** 15 standard deviations above mean ****

5. Q12238 Clone BR11 encoding PT-NANBH 834 198 198 15.37

**** 14 standard deviations above mean ****

6. Q12239 Clone 164/137 encoding PT-NAN 504 183 183 14.09

**** 11 standard deviations above mean ****

7. Q13146 PI HCV antigen. 225 153 171 11.55

**** 5 standard deviations above mean ****

8. Q11082 Encodes recombinant M. tubercu 1299 85 114 5.77

**** 4 standard deviations above mean ****

9. Q03259 Pseudorabies virus glycoprote 4897 70 111 4.50

10. N90002 Nucleotide Sequence of Densov 5957 70 101 4.50

11. Q05836 Human Brain Natriuretic Polyp 692 69 103 4.41

12. Q11495 Tobacco SURA-C3 mutant. 2520 69 111 4.41

13. N81459 C3 mutant gene encoding herbi 2520 69 111 4.41

14. Q11494 Tobacco SURB-Hra mutant. 2930 68 110 4.33

15. N81458 Hra mutant gene encoding herb 2946 68 110 4.33

16. N70607 Sequence encoding the melanom 2362 67 114 4.24

17. N60075 Sequence encoding human pancr 727 65 112 4.07

18. Q06001 Sequence encoding protein cha 12284 65 104 4.07

**** 3 standard deviations above mean ****

19. Q12569 Protease2 open reading frame 1209 64 107 3.99

20. Q12567 Alkaline protease cDNA from A 1513 64 107 3.99

Query sequence being compared: WORTMAN-616-FIG1 (1-240)

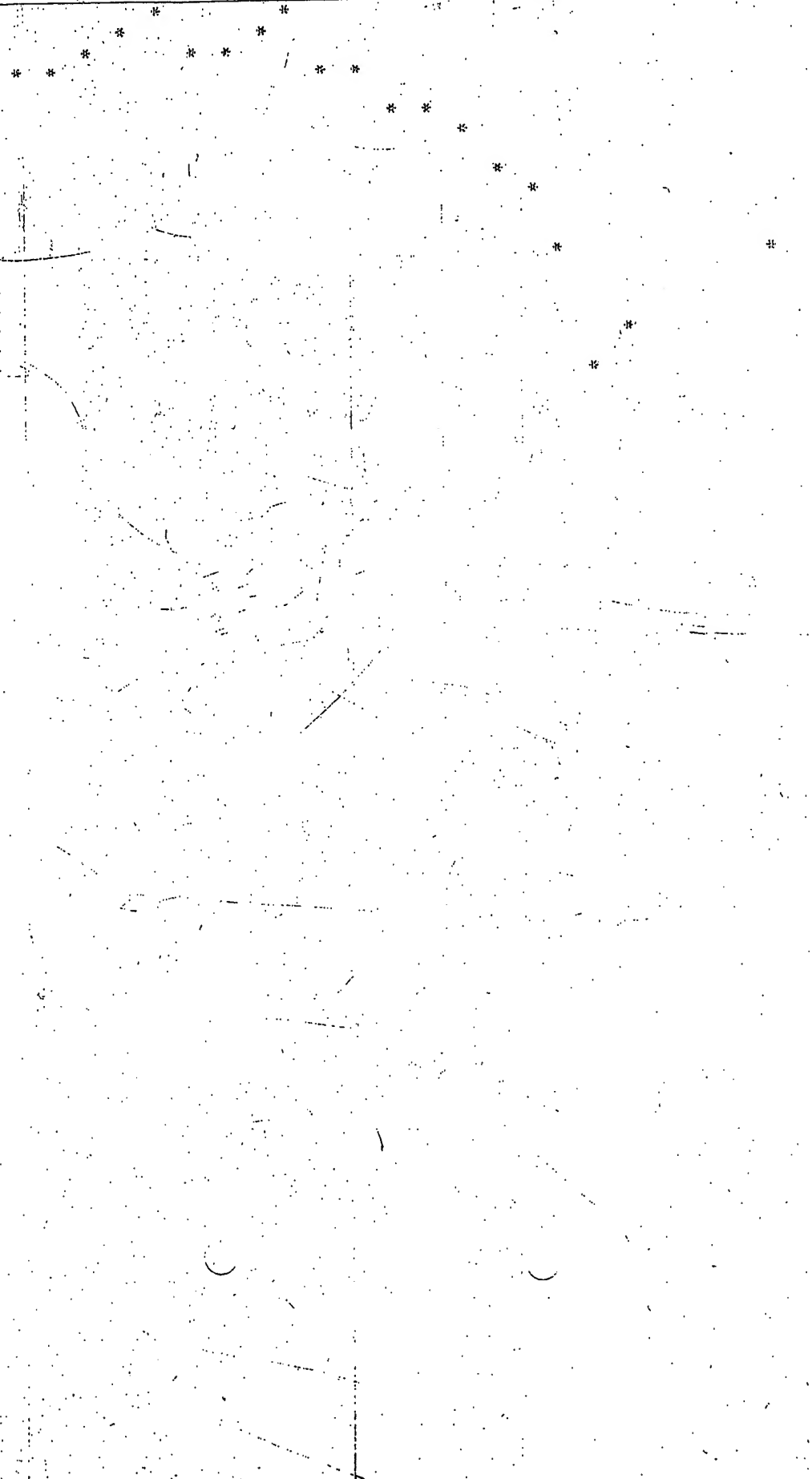
Number of sequences optimized: 3758

Results of the optimized comparison of WORTMAN-616-FIG1 (1-240) with:
Data bank : N-GeneSeq 5, all entries

30 1000-

N
U 500-
M35

B
E
R
Q40
F 100
S
E
Q45
U
E
N
C
E50
S



STDEV-3
SCORE

	-2	-1	0	1	2	3	4	5	6
SCORE	103	105	107	109	111	114	116	119	120

PARAMETERS

Similarity matrix	Unitary	K-tuple	4
Mismatch penalty	1	Joining penalty	30
Gap penalty	1.00	Window size	32
Gap size penalty	0.33		
Cutoff score	14		
Randomization group	0		

Initial scores to save	20	Alignments to save	10
Optimized scores to save	20	Display context	10

25 SEARCH STATISTICS

Scores:	Mean	Median	Standard Deviation
	107	110	2.43

Times:	CPU	Total Elapsed
	00:04:56.05	00:10:08.00

Number of residues: 6403247

Number of sequences optimized: 3758

35

The scores below are sorted by optimized score.
Significance is calculated based on optimized score.

A4000% identical sequence to the query sequence was not found.

The list of best scores is:

me	45 Sequence Name	Description	Length	Init. Score	Opt. Score	Sig. Fra	
	**** 52 standard deviations above mean ****						
0	1. Q10566	Hepatitis C virus strain 1 DN	9185	234	234	52.27	
0	2. Q05956	Sense strand of the compiled	9185	234	234	52.27	
0	50	**** 47 standard deviations above mean ****					
0	3. Q11075	Fragment of hepatitis C virus	552	220	223	47.74	
	**** 46 standard deviations above mean ****						

ID	Description	Size (bp)	Count	Score	Mean
4. Q12242	Encodes PT-NANBH viral struct	2116	220	220	46.51
**** 37 standard deviations above mean ****					
5. Q12238	Clone BR11 encoding PT-NANBH	834	198	198	37.45
**** 31 standard deviations above mean ****					
6. Q12239	Clone 164/137 encoding PT-NAN	504	183	183	31.28
**** 26 standard deviations above mean ****					
7. Q13146	P1 HCV antigen.	225	153	171	26.34
**** 6 standard deviations above mean ****					
8. Q03417	Plasmid pYUK121 contg. DNA enc	2070	56	123	6.59
**** 5 standard deviations above mean ****					
9. Q11374	Amyloid precursor protein pro	596	43	121	5.76
10. N90617	CD40 cDNA.	1004	29	121	5.76
11. N90323	Hepatitis C virus (HCV) cDNA	402	41	120	5.35
12. N92097	Combined open reading frames	5300	41	120	5.35
13. N90327	Hepatitis C virus composite p	5360	41	120	5.35
14. N92103	Combined open reading frames	6905	41	120	5.35
15. N92106	Combined open reading frames	7310	41	120	5.35
16. N90336	Composite hepatitis C virus (7310	41	120	5.35
17. Q05955	Hepatitis C virus open readin	8316	41	120	5.35
18. N92093	Sequence of the hepatitis C v	402	41	120	5.35
19. N70605	Plasmid pRF1 encoding rat liv	2450	27	120	5.35
**** 4 standard deviations above mean ****					
20. Q10503	6kB fragment contg. PHA polym	4500	53	119	4.94

125WORTMAN-616-FIG1 (1-240)

Q10566 Hepatitis C virus strain 1 DNA.

ID Q10566 standard; DNA; 9185 BP.
AC Q10566;
DE 29-APR-1991. (first entry)
DE Hepatitis C virus strain 1 DNA.
KW Hepatitis C virus; HCV-1; non-A, non-B hepatitis; HCV antigen;
KW viral infections; ss.
OS Hepatitis C virus.
BM EP-414475-A.
PD 27-FEB-1991.
PF 21-AUG-1990; 309120.
PR 25-AUG-1989; US-398667.
PA (CHIR-) CHIRON CORP.
RD Weiner AJ, Steimer S;
DR WPI; 91-059670/09.
DT Cell lines infected with hepatitis C virus - are used as source

PT of antigens for detection of HCV antibodies, vaccines, and
PT for screening anti-viral agents
AB Disclosure, fig 1; 4pp; English.
CC This is a hepatitis C virus (HCV) composite cDNA sequence, deduced
CC using overlapping clones. a compsn. contg. the antigenic protein
CC encoded by this sequence is useful for detecting anti-HCV anti-
CC bodies (Abs) and for screening an agent which inhibits HCV replic-
CC ation. A cell line infected with this virus can be used as a
CC source of antigens. The antigen is useful for preparing vaccines
CC for treating viral infections. See also Q10567.
SQ Sequence 9185 BP; 1849 A; 2790 C; 2608 G; 1938 T;

Initial Score = 234 Optimized Score = 234 Significance = 52.27
 Residue Identity = 97% Matches = 234 Mismatches = 6
 Gaps = 0 Conservative Substitutions = 0

```

      X      10      20      30      40      50      60
5      ATGAGCACGATTCCCAACCTCAAGGAAAAACCAACGTAACACCAACCGTCGCCACAGGA
      |||||
10     ACCGTGCACCATGAGCACGAATCCTAACCTCAAAAAAACAACGTAACACCAACCGTCGCCACAGGA
      |||||
15     CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTGCGCGCAGGGGCCCTAGATTGGG
      |||||
20     CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTGCGCGCAGGGGCCCTAGATTGGG
      |||||
25     TGTGCGCGCAGCAGGAGAACTTCCGAGCGGTGCGCAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGCACG
      |||||
30     TGTGCGCGCAGCAGGAGAACTTCCGAGCGGTGCGCAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGCACG
      |||||
35     TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
      |||||
40     TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
      |||||
45     TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
      |||||
50     TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
      |||||
55     TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
      |||||
60     TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
      |||||

```

230WORTMAN-616-FIG1 (1-240)

Q05956 Sense strand of the compiled Hepatitis C virus cDN

ID Q05956 standard; DNA; 9185 BP.
 AC Q05956;
 BS 23-JAN-1991 (first entry)
 DE Sense strand of the compiled Hepatitis C virus cDNA sequence.
 KW Hepatitis C virus (HCV); antiviral agent; ss.
 OS Hepatitis C virus.
 FH Key Location/Qualifiers
 FM CDS 320..9185
 FT /*tag= a
 FT misc_RNA 1..1667
 FT /*tag= b
 FT /note="epitope within this region is claimed"
 FT misc_RNA 8978..9185
 FT /*tag= c
 FT /note="encodes an epitope that is claimed"
 FN EP-388232-A.
 PD 19-SEP-1990.
 BZ 16-MAR-1990; 302866.
 PR 17-MAR-1989; US-325338.
 PR 20-APR-1989; US-341334.
 PR 18-MAY-1989; US-355002.
 PA (CHIR-) CHIRON CORP.

PI Houghton M, Choo QL, Kuo G;
 DR WPI; 90-284418/38.
 DR P-PSDB; R08124.
 PT Hepatitis C virus DNA - used for producing probes,
 PS polypeptide(s), antibodies and anti-sense polynucleotide(s) for
 PT diagnosis and therapy.
 PS Disclosure; Fig 17; 83pp; English.
 CC HCV cDNA libraries were constructed using pooled serum from a
 CC chimpanzee with chronic HCV infection. A lambda gt11 library was
 CC screened with probes derived from previously isolated clones. The
 CC ORF is derived from the overlapping clones b114a, ag30a, CA205a,
 CC CA290a, CA216a, pi4a, CA167b, CA156e, CA84a, CA59a, K9-1, 26j, 13i,
 CC 12f, 14i, 11b, 7f, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c,
 CC 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, 15e, b5a and 16jh. These
 CC clones extend the sequence of the HCV genome reported in EP-318216.
 CC The upstream region from nucleotides -319 to +1348 (=1-1667 in this
 CC file) is covered by clones b114a, 18g, ag30a, CA205a, CA290a,
 CC CA216a, pi4a, CA167b, CA156e, CA84a and CA59a; nucleotides
 CC 8659-8866 (=8978-9185 in this file) are covered by clones b5a and
 B0 16jh.
 CC See also 005955.
 SQ Sequence 9185 BP; 1849 A; 2790 C; 2605 G; 1938 T;

Initial Score = 234 Optimized Score = 234 Significance = 52.27
 Residue Identity = 97% Matches = 234 Mismatches = 6
 Gaps = 0 Conservative Substitutions = 0

```

      X      10      20      30      40      50      60
      ATGAGCAGCATTCCTCAACCTCAAGGAAAAACCAACGTACACCAACCGTCGCCACAGGA
30  |||||
      ACCGTGCACCATGAGCAGCATTCCTCAACCTCAAGGAAAAACCAACGTACACCAACCGTCGCCACAGGA
310 320 330 340 350 360 370 380

      70      80      90      100      110      120      130
35  CGTCAAGTTCCCGGGTGGCGGTCAGATCGTTGGTGGAGTTTACTTGTTGCCGCGCAGGGGCCCTAGATTGGG
      |||||
      CGTCAAGTTCCCGGGTGGCGGTCAGATCGTTGGTGGAGTTTACTTGTTGCCGCGCAGGGGCCCTAGATTGGG
390 400 410 420 430 440 450

      140      150      160      170      180      190      200
40  TGTGCGCGCGACGAGGAAAGACTTCCGAGCGGTTCGCAACCTCGAGGTAGACGTACGCTATCCCCAAGGCTCG
      |||||
      TGTGCGCGCGACGAGGAAAGACTTCCGAGCGGTTCGCAACCTCGAGGTAGACGTACGCTATCCCCAAGGCTCG
460 470 480 490 500 510 520

      210      220      230      X
45  TCAGCCCCAGGGGACGACCTGGGCTCAGCCCGGG
      |||||
      TCAGCCCCAGGGGACGACCTGGGCTCAGCCCGGGTACCCTTGGG
50 530 540 550 560
  
```

3. WORTMAN-616-FIG1 (1-240)
 Q11075 Fragment of hepatitis C virus J7 isolate C/E domain

ID Q11075 standard; DNA; 552 BP.
AC Q11075;
DT 30-MAY-1991 (first entry)
DE Fragment of hepatitis C virus J7 isolate C/E domain.
KE Hepatitis C virus; HCV-J1; HCV-J7; vaccines; NANBH; ss;
OS Hepatitis C virus.
FH Key Location/Qualifiers.
FT CDS 91..552
FT /*tag= a
EQ /label= HCV-J1 C/E domain
PN EP-419182-A.
PD 27-MAR-1991.
PF 17-SEP-1990; 310149.
PR 15-SEP-1989; US-408045.
PB 21-DEC-1989; US-456142.
PA (CHIR-) CHIRON CORP.
PI Miyamura T, Saito T, Houghton M, Weiner AJ, Han J;
PI Kolberg JA, Chata T-A, Irvine BD;
DR WPI; 91-088781/13.
BO P-PSDB; R11274.
PT New isolates J1 and J7 of hepatitis C virus - contg. specified
PT DNA and amino acid sequences; used in diagnosis, recombinant
PT protein prodn. and vaccine
PS Disclosure; fig 1; 109pp; English.
BB This is a fragment of the hepatitis C virus (HCV) J7 isolate C/E
CC domain. This is one of the domains of the viral isolate exhibiting
CC heterogeneity w.r.t the HCV1 isolate. This sequence has an imp-
CC ortant potential use as a probe in diagnostic assays and vaccine
CC development. Antibodies directed against it can be used for
CC screening antiviral agents and for isolation of non-A, non-B hepat-
CC itis (NANBH).
CC See also Q11076-79.
SQ Sequence 552 BP; 98 A; 161 C; 176 G; 103 T;
SQ 14 Others;
35
Initial Score = 220 Optimized Score = 223 Significance = 47.74
Residue Identity = 90% Matches = 223 Mismatches = 17
Gaps = 0 Conservative Substitutions = 0

```

40      X      10      20      30      40      50      60
      ATGAGCAGCATTCCCAACCTCAAGAGAAAAACCAACGTAACACCAACCGTCGCCACAGGA
      |||||
ACCGTGCAATCATGAGCACAATCCTAACCYSAAAGAAAAACCAACGTAACACCAACCGTCGCCACAGGA
      90      100      110      120      130      140      150

45      70      80      90      100      110      120      130
CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTTCGCCGCGAGGGGCCCTAGATTGGG
      |||||
CGTYAAGTTCCCGGGTGGCGGTGAGATCGTYGGTGGAGTTTACTTGTTCGCCGCGAGGGGCCCTAGATTGGG
      160      170      180      190      200      210      220

      140      150      160      170      180      190      200
TGTGCGCGCGAGGAGAGACTTCGAGCGGTGCGAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGACG
      |||||

```


TGTGCGTGCAGCTAGGAGAGACTTCCGAGCGGTCRCAACCTCGTGGAGGCGACACCTATCCCCAAGGCTCG
 230 240 250 260 270 280 290

210 220 230 X
 5 TCGGCCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
 |||
 CCGGCCCCGAGGGCAGGACCTGGGCTCAGCCTGGGTATCCTTGGC
 300 310 320 330 340

10
 4. WORTMAN-616-FIG1 (1-240)
 Q12242 Encodes PT-NANBH viral structural and non-structural

ID Q12242 standard; DNA; 2116 BP.
 OG Q12242;
 DT 17-SEP-1991 (first entry)
 DE Encodes PT-NANBH viral structural and non-structural proteins.
 KW post-transfusional non-A, non-B hepatitis; virus; vaccine; ss.
 OS Non-A, non-B hepatitis virus.
 EQ Key Location/Qualifiers
 FT CDS 308..2116
 FT /*tag= a
 PN GB2239245-A.
 PD 26-JUN-1991.
 RE 17-DEC-1990; 027250.
 PR 18-DEC-1989; GB-028562.
 PR 27-FEB-1990; GB-004414.
 PR 03-MAR-1990; GB-004814.
 PR 17-DEC-1990; GB-027250.
 SO (WELL) WELLCOME FOUNDATION LTD,
 PI Highfield PE, Rodgers BC, Tedder RS, Barbara JAJ;
 DR WPI; 91-187584/26.
 DR P-PSDB; R12600.
 PT Post-transfusional non-A non-B hepatitis poly:peptide(s) - and
 also DNA and antibodies used in diagnostic assays and in vaccines
 PS Claim 10; Page 83-87; 108pp; English.
 CC This sequence is thought to encode viral structural and non-
 structural proteins of the PT-NANBH viral genome which are antigenic.
 CC It was isolated from human serum infectious for the virus.
 SO See also Q12236-41.
 SO Sequence 2116 BP; 392 A; 650 C; 624 G; 450 T;

Initial Score = 220 Optimized Score = 220 Significance = 46.51
 Residue Identity = 91% Matches = 220 Mismatches = 20
 Gaps = 0 Conservative Substitutions = 0

X 10 20 30 40 50 60
 ATGAGCAGCAGATTCCCAACCTCAAGGAAAAACCAACCTAACACCAACCGTCGCCCCACAGGA
 |||
 50 ACCGTGCACCATGAGCAGCAGATTCCCAACCTCAAGGAAAAACCAACCTAACACCAACCGTCGCCCCACAGGA
 300 310 320 330 340 350 360
 70 80 90 100 110 120 130
 CGTCAAGTTCCCGGCTGGGTCAGATCGTTGGTGGAGTTACTTGTTCGCGCGAGGGGCCCTAGATTGGG


```

|||||
CGTCAAGTTCCCGGGCGGTGGTCAGATCGTTGGTGGAGTTTACCTGTTGCCGCGCAGGGGGCCCCACGTTGGG
370      380      390      400      410      420      430      440

5      140      150      160      170      180      190      200
TGTGGGGGCGACGAGGAAGACTTCCGAGCGGTCGCAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGCACG
|||||
TGTGCGCGCGACTAGGAAGACTTCCGAGCGGTCGCAACCTCGTGGAGGGCGACAACCTATCCCCAAGGCTCG
450      460      470      480      490      500      510

10      210      220      230
TCGGCCCCGAGGGGCGAGGACCTGGGCTCAGCCCCGGG
|||||
CCAGCCCCGAGGGGCGAGGCTGGGCTCAGCCCCGGGTACCCTTGGC
15      520      530      540      550

```

5. WORTMAN-616-FIG1 (1-240)
Q12238 Clone BR11 encoding PT-NANBH virus antigenic porti

ID Q12238 standard; DNA; 834 BP;
AC Q12238;
DT 06-SEP-1991 (first entry)
DE Clone BR11 encoding PT-NANBH virus antigenic portion.
EQ post-transfusional non-A, non-B hepatitis; virus; vaccine; 55.
OS Non-A, non-B hepatitis virus.
PN Q82239245-A.
PD 26-JUN-1991.
PF 17-DEC-1990; Q27250.
Q8 18-DEC-1989; GB-Q28562.
PR 27-FEB-1990; GB-Q04414.
PR 03-MAR-1990; GB-Q04614.
PR 17-DEC-1990; GB-Q27250.
PA (WELL) WELLCOME FOUNDATION LTD.
Q5 Highfield PE; Rodgers BC, Tedder RS, Barbara JAJ;
DR WPI; 91-187584/26.
DR P-PSDB; R12596.
PT Post-transfusional non-A non-B hepatitis poly:peptide(s) - and
PT also DNA and antibodies used in diagnostic assays and in vaccines
EQ Claim 10; Page 56-58; 108pp; English.
CC This sequence is a structural region of the PT-NANBH viral
CC genome encoding an antigenic polypeptide. It was isolated from serum
CC A cDNA library was prepared in lambda gt11 from the serum of infected
CC patients and screened with antibodies from the serum of humans with a
EQ high risk for PT-NANBH but which did not react with viral antigens
CC DX113, BHC-5 and BHC-7. Clone BR11 was identified which did not
CC cross-hybridise with probes made from JG2 and JG3 (see Q12236 and
CC Q12237). It was sequenced and found to have the sequence shown, which
CC includes the EcoRI linkers added during cloning.
Q8 See also Q12239-Q12242.
Q8 Sequence 834 BP; 139 A; 265 C; 251 G; 179 T;

Initial Score = 198 Optimized Score = 198 Significance = 37.45
Residue Identity = 91% Matches = 198 Mismatches = 18

17

WORTMAN-616-FIG1 (1-240)

Q12239 Clone 164/137 encoding PT-NANBH virus antigenic po

```

30
ID      Q12239 standard; DNA; 504 BP.
AC      Q12239;
DT      06-SEP-1991 (first entry)
DE      Clone 164/137 encoding PT-NANBH virus antigenic portion.
EM      post-transfusal non-A, non-B hepatitis; virus; vaccine; ss.
OS      Non-A, non-B hepatitis virus.
FH      Key Location/Qualifiers
FT      CDS 308..504
FT      /*tag= a
FM      /product= start of PT-NANB polyprotein
PN      GB2239245-A.
PD      26-JUN-1991.
PF      17-DEC-1990; 027250.
PR      18-DEC-1989; GB-028562.
PR      27-FEB-1990; GB-004414.
PR      03-MAR-1990; GB-004814.
PR      17-DEC-1990; GB-027250.
PA      (WELL ) WELLCOME FOUNDATION LTD.
PI      Highfield PE, Rodgers BC, Tedder RS, Barbara JAJ;
BO      WPI; 91-187584/26.
DR      P-PSDB; R12597
PT      Post-transfusal non-A non-B hepatitis poly:peptide(s) - and
PT      also DNA and antibodies used in diagnostic assays and in vaccines
PS      Claim 10; Page 71-72; 108pp; English.

```


CC This sequence probably encodes viral structural proteins of the
 CC PT-NANBH viral genome having antigenic properties. It was
 CC isolated from serum of humans infected by the virus.
 CC See also Q12236-F8 and Q12240-Q12242.
 SB Sequence 504 BP; 106 A; 147 C; 153 G; 98 T;

Initial Score = 183 Optimized Score = 183 Significance = 31.28
 Residue Identity = 92% Matches = 183 Mismatches = 14
 Gaps = 0 Conservative Substitutions = 0

```

10      X      10      20      30      40      50      60
      ATGAGCAGGATTCCCAAACTCAAAGAAAACCAAAACGTAAACCAACCGTCGCCACAGGA
      ||||| ||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
15 300 310 320 330 340 350 360
      70      80      90      100     110     120     130
      CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTTCGCCGCGAGGGGCCCTAGATTGGG
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
20 370 380 390 400 410 420 430 440
      140     150     160     170     180     190     200
      TGTGCGCGCGAGGAGAGACTTCGAGCGGTCCGAACCTCGAGGTAGACGTACGCTATCCCAAGGCACG
25 T ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
      TGTGCGCGCGAGTAGGAGAGACTTCGAGCGGTCCGAACCTCGTGGAGGCGGACACCTATCCC
      450     460     470     480     490     500     X
  
```

30 T

7. WORTMAN-616-FIG1 (1-240)

Q13146 P1 HCV antigen.

35 ID: Q13146 standard; DNA; 225 BP.
 AC: Q13146;
 DT: 23-OCT-1991 (first entry)
 DE: P1 HCV antigen.
 MO: C100-3; hepatitis C virus; immunoassay; epitope; ss.
 OS: Synthetic.
 PN: AU9068390-A.
 PD: 27-JUN-1991.
 PF: 21-DEC-1990; 068390.
 RD: 22-DEC-1989; US-456162.
 PR: 07-NOV-1990; US-610180.
 RA: (ABBO) ABBOTT LABORATORIES.
 DR: WPI; 91-238393/33.
 DR: P-PSDB; R13343.
 SO: Immunological assays for hepatitis C virus antibody - by using
 PT: polypeptide(s) contg. epitope(s) of hepatitis C virus antigens
 PS: Disclosure; Page 20; 62pp; English.
 CC: The polypeptide used in the improved immunological assay may be
 CC: prepared using recombinant technology. The amino acid sequence for

CC p1 is reverse translated to give the codons represented here which
 CC are optimised to facilitate high level expression in E. coli.
 CC Individual oligonucleotides are synthesised and annealed and ligated
 CC together to assemble the entire DNA sequence for digestion with
 CC BamHI and SalI, allowing ligation into pUC18. The resulting plasmid
 CC is suitably transformed into E. coli JM103 cells.
 CC The assay has increased sensitivity and is more specific than
 CC assays using the polypeptide C100-3 (EP-318216).
 CC See also Q13146-48 and R13343-65.
 CC Sequence 225 BP; 48 A; 67 C; 61 G; 49 T;

Initial Score = 153 Optimized Score = 171 Significance = 26.34
 Residue Identity = 76% Matches = 171 Mismatches = 54
 Gaps = 0 Conservative Substitutions = 0

```

15 X      10      20      30      40      50      60      70
   ATGAGCAGCATTCCCAACCTCAAAGAAAAACCAACGTAACACCAACCGTCGCCACAGGACGTCAAGTTC
   ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
   ATGTCTACCAACCCGAAACCGCAGAAAAAAACCAACGTAACACCAACCGTCGTCGCGAGGACGTTAATTTC
20 X      10      20      30      40      50      60      70
      80      90      100     110     120     130     140
   CCGGGTGGCGGTTCAGATCGTTGGTGGAGTTTACTTGTTCGCCGCGAGGGGCCCTAGATTGGGTGTGCGCGCG
   ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
25 CCGGGTGGGTTCAGATCGTTGGTGGTGTTCCTGCTGCCGCGTCGIGGTCCGCGTCTGGGTGTTCTGTGCT
   80      90      100     110     120     130     140
      150     160     170     180     190     200     210
   ACBAGGAAGACTTCCGAGCGGTTCGCAACCTCGAGGTAGACGTCAGCCTATCCCAAGGCACGTCGGCCCCGAG
30 ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
   ACCCGTAAACCTCTGACGTTCTCAGCCGCGTGGTTCGTCGTCAGCCGATCCCGAAAGCTCGTCGTCGGGAA
      150     160     170     180     190     200     210

```

```

220 X 230
35 GGCAGGACCTGGGCTCAGC
   ||| |||
   GGTCTGACC
220 X

```

40

45

50

> O (<
OI IO IntelliGenetics
> O (<

FastDB - Fast Pairwise Comparison of Sequences
Release 5.4

Results file wortman-616-fig1.res made by maryh on Wed 25 Mar 92 12:47:31-PST

10
Query sequence being compared: WORTMAN-616-FIG1 (1-240)
Number of sequences searched: 65202
Number of scores above cutoff: 4191

15 Results of the initial comparison of WORTMAN-616-FIG1 (1-240) with:
Data bank : EMBL-NEW 1, all entries
Data bank : GenBank 70, all entries
Data bank : GenBank-NEW 1, all entries
Data bank : UEMBL 29_70, all entries

20
100000-

N
U50000-
M25
B
E
R

030
F10000-

S
E 5000-
035
U
E
N
C
E40
S 1000-*

500-
45

50
100-
50-

**

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*

Cut-off raised to 40.

The scores below are sorted by initial score.
Significance is calculated based on initial score.

A 100% identical sequence to the query sequence was not found.

The list of best scores is:

Sequence Name	Description	Length	Init. Score	Opt. Score	Sig. Fra
**** 22 standard deviations above mean ****					
1. HPCST77	Hepatitis C virus structural	2610	238	238	22.25
2. HPCCGAA	Hepatitis C virus, complete g	9416	238	238	22.25
3. HPCST90	Hepatitis C virus structural	2610	236	236	22.04
**** 21 standard deviations above mean ****					
4. HPCPLYPRE	Hepatitis C virus polyprotein	9401	234	234	21.83
**** 20 standard deviations above mean ****					
5. HPCCHUMR	Hepatitis C virus core, matri	9416	223	223	20.69
6. HCVJK1	Hepatitis C virus (JK1) core,	2672	222	222	20.59
7. HPCVJK1	Hepatitis C virus (JK1) core,	2672	222	222	20.59
8. HCVJK1	Hepatitis C virus (JK1) core,	2672	222	222	20.59
9. HPCCORE1	Hepatitis C virus core protei	534	221	221	20.48
10. HCVJK1G	Hepatitis C virus core, E1, N	9408	221	221	20.48
11. HPCVJK1G	Hepatitis C virus core, E1, N	9408	221	221	20.48
12. HCVJK1G	Hepatitis C virus core, E1, N	9408	221	221	20.48
13. HPCJCG	Hepatitis C virus (HCV-J) com	9413	221	221	20.48
14. HCVJK2	Hepatitis C virus (JK2) core,	2672	220	220	20.38
15. HPCVJK2	Hepatitis C virus (JK2) core,	2672	220	220	20.38
16. HCVJK2	Hepatitis C virus (JK2) core,	2672	220	220	20.38
17. HPCCORE2	Hepatitis C virus core protei	534	219	219	20.27
18. HCVJK4	Hepatitis C virus (JK4) core,	2672	219	219	20.27
19. HPCVJK4	Hepatitis C virus (JK4) core,	2672	219	219	20.27
20. HCVJK4	Hepatitis C virus (JK4) core,	2672	219	219	20.27

Query sequence being compared: WORTMAN-616-FIG1 (1-240)

Number of sequences optimized: 4191

40

Results of the optimized comparison of WORTMAN-616-FIG1 (1-240) with:

Data bank : EMBL-NEW 1, all entries

Data bank : GenBank 70, all entries

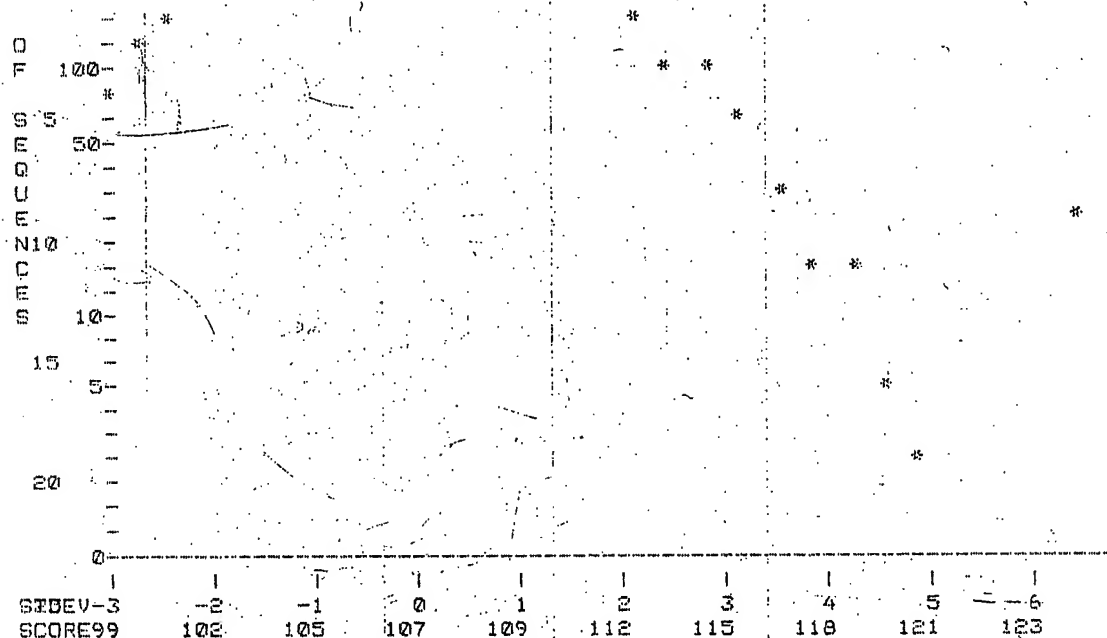
Data bank : GenBank-NEW 1, all entries

45 Data bank : UEMBL 29_70, all entries

1000-

N
US0500-
M
B
E
R

* * * * *
* * * * *



PARAMETERS

30
 Similarity matrix Unitary K-tuple 4
 Mismatch penalty 1 Joining penalty 30
 Gap penalty 1.00 Window size 32
 Gap size penalty 0.33
 Cutoff score 14
 Randomization group 0

Initial scores to save 20 Alignments to save 10
 Optimized scores to save 20 Display context 10
 40

SEARCH STATISTICS

Scores: Mean Median Standard Deviation
 107 111 2.98

45
 Times: CPU Total Elapsed
 00:06:19.95 00:13:52.00

Number of residues: 12567488
 Number of sequences optimized: 4191

The scores below are sorted by optimized score.
 Significance is calculated based on optimized score.

A 100% identical sequence to the query sequence was not found.

The list of best scores is:

5

me	Sequence Name	Description	Init. Opt.		Sig. Fra
			Length	Score	
		**** 45 standard deviations above mean ****			
10	1. HPCST77	Hepatitis C virus structural	2610	238	45.52
0	2. HPCCGAA	Hepatitis C virus, complete g	9416	238	45.52
		**** 44 standard deviations above mean ****			
0	3. HPCST90	Hepatitis C virus structural	2610	236	44.82
0	4. HPCPLYPRE	Hepatitis C virus polyprotein	9401	234	44.13
15	5. HPCHLMR	Hepatitis C virus core, matri	9416	223	40.30
0		**** 39 standard deviations above mean ****			
0	6. HCVJK1	Hepatitis C virus (JK1) core,	2672	222	39.96
0	7. HPCVJK1	Hepatitis C virus (JK1) core,	2672	222	39.96
0	8. HCVJK1	Hepatitis C virus (JK1) core,	2672	222	39.96
20	9. HPCCDRE1	Hepatitis C virus core protei	534	221	39.61
0	10. HCVJK1G	Hepatitis C virus core, E1, N	9408	221	39.61
0	11. HPCVJK1G	Hepatitis C virus core, E1, N	9408	221	39.61
0	12. HCVJK1G	Hepatitis C virus core, E1, N	9408	221	39.61
0	13. HPCJCG	Hepatitis C virus (HCV-J) com	9413	221	39.61
25	14. HCVJK2	Hepatitis C virus (JK2) core,	2672	220	39.26
0	15. HPCVJK2	Hepatitis C virus (JK2) core,	2672	220	39.26
0	16. HCVJK2	Hepatitis C virus (JK2) core,	2672	220	39.26
0	17. HPCRNASP	Hepatitis C virus RNA, 5'-non	1413	217	39.26
30		**** 38 standard deviations above mean ****			
0	18. HCVJK4	Hepatitis C virus (JK4) core,	2672	219	38.91
0	19. HPCVJK4	Hepatitis C virus (JK4) core,	2672	219	38.91
0	20. HCVJK4	Hepatitis C virus (JK4) core,	2672	219	38.91

35

HPCST77 Hepatitis C virus structural gene, 5' end.

LOCUS HPCST77 2610 bp ss-RNA VRL 02-APR-1991
DEFINITION Hepatitis C virus structural gene, 5' end.
ACCESSION M62381
KEYWORDS structural gene.
SOURCE Hepatitis C virus (Human patient 'H') 1977 isolate, RNA.
ORGANISM Hepatitis C virus
45 Viridae; ss-RNA enveloped viruses; Positive strand RNA virus;
Togaviridae incertae sedis.
REFERENCE 1 (bases 1 to 2610)
AUTHORS Ogata, N., Alter, H. J., Miller, R. H. and Purcell, R. H.
TITLE Nucleotide sequence and mutation rate of the H strain of hepatitis
is C virus
50 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 88, 3392-3396 (1991)
STANDARD simple staff entry
FEATURES Location/Qualifiers
CDS 1816..>2610

/codon_start=1

/translation="MVVGTDRSGAPTYSWGANDTDVFLNNTRPPLGNWFGCTWMNS

TG6TKVCGAPPCVIG6V6NNTLLCPTDCFRKHPEATYSRC666PWITPRCMVDYPYRL

WHYPCTINYTIFKVRMYV66VEHRLEAACNWRGERCDLEDRDSELSPLLLSTTQWQ

VLPCSFTTLPALSTGLIHLQNIQVQYLY6V6SSIASWAIKWEYVLLFLLADARV

10 C S C L W M M L L I S Q A E A L E N L V I L N A A S L A G T H G L V S F L V F F C F A W Y L "

BASE COUNT 483 a 786 c 763 g 578 t
ORIGIN

Initial Score = 238 Optimized Score = 238 Significance = 45.52
Residue Identity = 99% Matches = 238 Mismatches = 2
Gaps = 0 Conservative Substitutions = 0

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20
ACCGTGCACCATGAGCACGAATCCTAACCTCAAGAGAAAACCAACGTACACCAACCGTCGCCACAGGA
270 280 290 300 310 320 330
70 80 90 100 110 120 130
25 CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTGGCCGCGCAGGGGCCCTAGATTGGG
CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTGGCCGCGCAGGGGCCCTAGATTGGG
340 350 360 370 380 390 400 410
30 140 150 160 170 180 190 200
TGTGCGCGCGACGAGGAAAGACTTCCGAGCGGTGCGAAGCTCGAGGTAGACGTACGCTATCCCCAAGGCACG
TGTGCGCGCGACGAGGAAAGACTTCCGAGCGGTGCGAAGCTCGAGGTAGACGTACGCTATCCCCAAGGCACG
420 430 440 450 460 470 480
35 210 220 230 240
TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGGTACCTTGGC
40 490 500 510 X 520

2. WORTMAN-616-FIG1 (1-240)

HPCCGAA Hepatitis C virus, complete genome.

45
LOCUS HPCCGAA 9416 bp ss-RNA VRL 07-NOV-1991
DEFINITION Hepatitis C virus, complete genome.
ACCESSION M67463
KEYWORDS complete genome; polyprotein.
SOURCE Hepatitis C virus cDNA to mRNA.
ORGANISM Hepatitis C virus
Viridae; ss-RNA enveloped viruses; Positive strand RNA virus;
Togaviridae; incertae sedis.
REFERENCE 1 (bases 1 to 9416)

AUTHORS Inchauspé, G., Zebedee, S., Lee, D.-H.H., Sugitani, M., Nasoff, M. and

TITLE Prince, A.M.
Genomic structure of the human prototype strain H of hepatitis C
virus: Comparison with American and Japanese isolates
5 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 88, 10292-10296 (1991)
STANDARD full staff entry
FEATURES Location/Qualifiers
CDS 342..9377
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GVNYATGNLPGCSFSIFLLAELSLTVPASAYQVRNSSGLYHVTNDCPNSSVVEAAD
AIDHTPGCVPCVREGNASRCWAVTPTVATRDGKLPTTLRRHIDLLVGSATLCSALY
VGDLGGSVFLVGQLFTFSRHHWTTQDCNCSIYPGHITGHRMAWNMMMNWSPTAALVV
AQLLRIPQAIMDMIAGAHWGLAGIKYFSMVGNWAKVLVLLLFAGVDAETHVTGGNA
25 GRTTAGLVGLLTPGAKNIQLINTNGSWHINSTALNCNESLNTGWLGLFYQHKFNSS
GCPERLASCRRLTDFAGWQPISYANGSGLDERPYCWHYPPRPGGIVPAKSVCSPVYC
FIDSPVVVGTTDRSGAPTYSWGANDTDVFLNNTRPPLGNWFGCTWMNSTGFTKVCGA
PPCVIGGVGNNTLLCPTDCFRKYPEATYSRCGSGPRITPRCMVDYPYRLWHYPCTINY
TIFKVRMYVGGVEHRLAACNWRGERCDLEDNRSELSPLLLSTGQVLPQSFITL
35 PALSTGLIHLHQNIVDVQYLYGVSSIASWAIKWEYVLLFLLLADARVCSCLWMMLL
ISGAEALENLVILNAAAGLTHGLVSFLVFFCFAYLKGWVPGAVYALYGMWPLLL
LQDALPQRAYALDEVAASCGGVVLVGLMALTLSPYKRYISWCMWWLQYFLTRVEAQ
LHVWVPPLNVRGGDAVILLTCVHPALVFDITKLLAIFGPLWILQASLLKVPYFVR
VQGLLRICALARKIAGGHYVQMAIKLGALTGTCTVYNHLAPLRDWAHNGLRDLAYAVE
45 PVVFSRMETKLITWADTAACGDIINGLPVSARRGQELLGPADGMVSKGWRLAPIT
AYAQQTRGLLGCIITSLTGRDKNQVEGEVQIVSTATQTELATCINGVCWTVYHGAGTR
TQSPKGPVIQTYTNVDQDLVGW GSRSLTPCTCGSSDLYLVTRHADVII IRRG

DSRGSLSPRPISYLGSSGGPILLCPTGHAVGLFRAAVCTRGVAKAVDFII NLETT

MREPVFTDNSSPPAVFGSFQVAH. TSGKSTKVPAAYAAGYKVLVLNP ATL

GFGAYMSKAHQVDENIRTVRTITTGSPITYSTYCKFLADAGCSGGAYDIIICDECHS
TDATSISSIGTGLDQAETAGARLVVLATATPPGSVTVSHPNIEEVALSTTGEIPFYGK
5
AIPLEVIKGRALIFCHSKKKCDELAACKLVALGINAVAYYRGLDVSUIPTSGDVVVVS
TDALMTGFTGDFDSVIDCNTCTGTVDLSLDPFTTETTTLPQDAVSRTQRRGRTGRS
KROIRFVAPBERPSGMFDSSVLCEDYAGCAWYELTPAETTURLRAYMNTPLPVCG
DHLGFWEGVFTGLTHIDAHFLSQTKQSGENFPYLVAQGATVCARAQAPPASWDQMRKC
LIRLKPTLHGPTPLLYRLGAVQNEVTLTHPITKYIMTCMSADLEVVTSTWVLVGGVLA
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QFKQKALGLLQTSRHAEVITPAVQTNWQKLEVFwakHMMWFISGIGYLAGLSTLFGN
PQASLMAFTAAVTSPLTTGQTLLENILGGWVAQLAAPGAATAFVBAQLAGAALDSV
GLGKVLVDILAGYGAGVAGALVAFKIMSBEVPSTEDLVNLLPAILSPBALAVGVVFAS
ILRRRVGPGEGAVQWMNRLIAFASRGNHVSPTHYVPESDAAARVTAISSLTVTQLLR
25
RLHQWISSECTTPCSGSLWRDIWDWICEVLSDFKTWLKAKLMPQLPGIPFVSCQGRYR
GVWRGDBIMHTRCHGBAEITGHVKNGTMRIVGPRCKNMWSGTEFFINAYTTGPCTPLP
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FAPPCKPLLREEVSFRVGLHEYPVGSQLPCEPEPDVAULTSMLTDPSHITAEAGRRL
ARGSPPSMASSASQLSAPSLKATCTANHDSPPDAELIEANLLWRQEMGGNITRVESEN
35
KVVILDSFDALVAEEDEREVSVPRAELRKSRRFAPALPVWARPDYNPLLVEWKKPDY
EPPVHGCPLPPRSPVPVRKRTVVLTESTLPTALAEATKSGSGSSTSGITQDN
TMSSEPAPSGCPPDSVSESYSMPLEGEPPDLSGGSWSTVSSGADTEDVVCOSM
SYSWTBALVTPCAEEGKLPINALSNSLLRHHNLVYSTTSRSACQKKKVTFDRLQVL
DSHYQDVLKEVKAASKVKANLLSVEEACSLAPPHSAKSKFGYGAKDVRCHARKAVAH
45
INSVWKDLLEDSTPIDTTIMAKNEVFCVQPEKGGKPAARLIVFPDLGVRVCEKMALY
DVVSKLPLAVMGSSYGFGYSPGQVEFLVQAWKSKKTPMGLSYDTRCFDSTVTESDIR
TGBAIYQCCDLDPQARVAIKSLTERLYVGGPLTNSRGENCYARRCRASRVLTTSQNT
LTRYIKARAACRAAGLQDCTMLVCGDDLUVVICESAGVQEDAASLRAFTEAMTRYBAPP
GDFPQPEYDLELITSCSSNVSAHDGAGKRVYYLTRDPTTPLARAQWETARHTPVNSW

LGNIIMFAPTLWARMILMTHFFSVLIARDQLEALNCEIYGACYSIEPLDLPPIIQR

HGLSAFSLH8YSPGEINRVAACLRLKLGVPPLRAWRHRAVSURARLLARGGKAAICGKY

5

LFNWAVRTKLKLTPTAABRLDLGWFAGYSGSDIYH8VSHARPRWFCLLLAAG

VGIIYLLPNR"

BASE COUNT 1880 a 2854 c 2684 g 1999 t

ORIGIN

10

Initial Score = 238 Optimized Score = 238 Significance = 45.52

Residue Identity = 99% Matches = 238 Mismatches = 2

Gaps = 0 Conservative Substitutions = 0

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|||||
ACCGTGCACCATGAGCAGGATTCCTCAACCTCAAGAAAAACCAACCTAACACCAACCGTCGCCCCACAGGA
340 X 350 360 370 380 390 400
20 70 80 90 100 110 120 130
CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTGCGCGCAGGGGCCCTAGATTGGG
|||||
CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTGCGCGCAGGGGCCCTAGATTGGG
25 410 420 430 440 450 460 470
140 150 160 170 180 190 200
TGTGCGCGCAGGAGGAGACTTCCGAGCGGTGCGAACCTCGAGGTAGACGTAGCCTATCCCCAAGGCACG
|||||
30 TGTGCGCGCAGGAGGAGACTTCCGAGCGGTGCGAACCTCGAGGTAGACGTAGCCTATCCCCAAGGCACG
480 490 500 510 520 530 540
210 220 230 X
TCGCCCCGAGGGCAGGACCTGGGCTCAGCCCCGGG
35 |||||
TCGCCCCGAGGGCAGGACCTGGGCTCAGCCCCGGGTACCCTTGGC
550 560 570 580 590

340WORTMAN-616-FIG1 (1-240)

HPCST90 Hepatitis C virus structural gene, 5' end.

LOCUS HPCST90 2610 bp ss-RNA URL 02-APR-1991
DEFINITION Hepatitis C virus structural gene, 5' end.
ACCESSION M62382
KEYWORDS structural gene.
SOURCE Hepatitis C virus (human patient 'H') 1990 isolate viral RNA.
ORGANISM Hepatitis C virus
Viridae; ss-RNA enveloped viruses; Positive strand RNA virus;
Togaviridae incertae sedis.
50 REFERENCE 1 (bases 1 to 2610)
AUTHORS Ogata, N., Alter, H.J., Miller, R.H. and Purcell, R.H.
TITLE Nucleotide sequence and mutation rate of the H strain of hepatitis
is C virus

JOURNAL Proc. Natl. Acad. Sci. U.S.A. 88, 3392-3396 (1991)
STANDARD simple staff_entry

FEATURES Location/Qualifiers
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10
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VLPCSFITLPAALSTGLIHLHQMIVDVQVLYGVGSSIASWTIKWEYVVLFLLLADARV
CSCLWMMLLISQAEAALENLVILNAASLAGAHGLVSFLVFFCFAYL"

BASE COUNT 490 a 784 c 759 g 577 t
ORIGIN

Initial Score = 236 Optimized Score = 236 Significance = 44.82
Residue Identity = 98% Matches = 236 Mismatches = 4
Gaps = 0 Conservative Substitutions = 0

```

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      |||||
25 ACCGTGCATCATGAGCAGCAATCCTAAACCTCAAGAAAAACCAACGTAAACACCAACCGTCGCCACAGGA
   270   280   290   300   310   320   330
      70      80      90     100     110     120     130
      CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTTCGCCGCGAGGGGCCCTAGATTGGG
30 |||||
      CGTCAAGTTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTTCGCCGCGAGGGGCCCTAGATTGGG
   340   350   360   370   380   390   400   410
      140     150     160     170     180     190     200
35 TGTGCGCGCGACGAGGAAGACTTCCGAGCGGTGCGAAGCTCGAGGTAGACGTAGCCTATCCCCAAGGCACG
      |||||
      TGTGCGCGCGACGAGGAAGACTTCCGAGCGGTGCGAAGCTCGAGGTAGACGTAGCCTATCCCCAAGGCACG
   420   430   440   450   460   470   480
      40     210     220     230     240
      TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCCGGG
      |||||
      TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCCGGGTACCTTGGC
   490   500   510   X 520
45
```

4. WORTMAN-616-FIG1 (1-240)

HPCPLYPRE Hepatitis C virus polyprotein precursor (HCV-1) mRNA

LOCUS HPCPLYPRE 9401 bp ss-RNA VRL 08-MAY-1991
DEFINITION Hepatitis C virus polyprotein precursor (HCV-1) mRNA, complete
cds.
ACCESSION M62321
KEYWORDS HCV-1 polyprotein precursor.

SOURCE Hepatitis C virus, cDNA to viral RNA.
 ORGANISM Hepatitis C virus.
 Viridae; ss-RNA enveloped viruses; Positive strand RNA virus;
 Togaviridae incertae sedis.
 REFERENCE 1 (bases 1 to 9401)
 AUTHORS Choo, Q.-L., Richman, K., Han, J.H., Berger, K., Lee, C., Dong, C.,
 Gallegos, C., Coit, D., Medina-Selby, A., Barr, P.J., Weiner, A.J.,
 Bradley, D.W., Kuo, G. and Houghton, M.
 TITLE Genetic organization and diversity of the hepatitis C virus
 10JOURNAL Proc. Natl. Acad. Sci. U.S.A. 88, 2451-2455 (1991)
 STANDARD simple staff_entry
 FEATURES Location/Qualifiers
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 /codon_start=1

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 GVNATONLPGCSFSIFLLALLSCLTPASAYQVRNSTGLYHVTNDCPNSSIVYEAD
 AIEHTPGCVPCVREGNASRCWVAMTPTVATRDGKLPATQLRRHIDLLVGSATLCSALY
 VGDLCGSVFLVGQLFTFSRRHWTTGNCNSIYPGHITGHRMAWDMMNWSPITALVM
 AQLLRIPQAILDMIAGAHWGLAGIAYFSMVGNWAKVLVULLFAGVDAETHVTGGSA
 30 GHTVSGFVSLLAPGAKQNVQLINTNGSWHLNSTALNCNDSLNTGWLAGLFYHHKFNS
 GCPERLASCRPLTDFDQGWGPISYANGSGPDQRFYCWHYPPKPCGIVPAKSVCGPVYC
 FEBSPVVGTTRSGAPTYSWGENDTDVFLNNTRPFLGNWFGCTWMNSTGFTKVCBA
 PPCVIGGAGNNTLHCPTDCFRKHPDATYSRCGSGPWITPRCLVDYPYRLWHYPCTINY
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 LHVWIPPLNVRGGDAVILLCAVHPTLVFDITKLLAVFGPLWILDASLLKVPYFVR
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 50 PVVFSQMETKLITWGADTAACQDIINGLPVSARRGREILLGPADGMVSKGWRLAPIT
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TIASPKQPVIGMYTNVDQDLVGWPAFQGSRLTPCTCGSSDLYLVTRHADVIPVRRRG
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5
MRSPVFTDNSSPPVVPQSFQVAHLHAPTGSBKSTKVPAAYAAGGYKVLVLPNSVAATL
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ARQSPPSVASSASQLSAPSLKATCTANHDSFDAELIEANLLWRQEMGGNITRVESEN
KVVILDSFDPLVAEEDEREISVPAEILRKSRRFAQALPVWARPDPNPLVETWKKPDY
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45
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DSBYQDVLEKVKAAASKVKANLLSVEEACSLTPHSAKSKFGYGAKDVCHARKAVTH
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TEEAIYQCCDLDPQARVAIKSLTERLYVGGPLTNSRGENCYRRCRASQVLTTSQNT
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 5
 QDPQPEYDLELITSCSSNVSVANDGAGKRVYYLTRDPTPLARAAWETARHTPVNSW
 LGNIIMFAPTLWARMILMTHFFSVLIARDQLEQALDCEIYGACYSIEPLDLPPIIQR
 HDBSAFSLHSYSPGEINRVAACLRLKLGVPPLRAWHRARSVRARLLARGGAAICGKY
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 VGIYLLPNR"

BASE COUNT 1883 a 2860 c 2673 g 1985 t
 ORIGIN

Initial Score = 234 Optimized Score = 234 Significance = 44.13
 Residue Identity = 97% Matches = 234 Mismatches = 6
 Gaps = 0 Conservative Substitutions = 0

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      70      80      90      100      110      120      130
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      |||||
30  CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTTGCCGCGCAGGGGCCCTAGATTGGG
      410      420      430      440      450      460      470
      140      150      160      170      180      190      200
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      |||||
35  TGTGCGCGCAGCAGGAGAGACTTCCGAGCGGTGCGAACCTCGAGGTAGACGTACGCCTATCCCCAAGGCTCG
      480      490      500      510      520      530      540
      210      220      230      X
40  TCGGCCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
      |||||
      TCGGCCCCGAGGGCAGGACCTGGGCTCAGCCCGGGTACCCCTTGEC
      550      560      570      580      590

```

45
 5. WORTMAN-616-FIG1 (1-240)
 HPCHUMR Hepatitis C virus core, matrix, envelope and non-s

LOCUS HPCHUMR 9416 bp ss-RNA VRL 22-MAR-1991
 DEFINITION Hepatitis C virus core, matrix, envelope and non-structural
 protein
 RNA.
 ACCESSION M58335
 KEYWORDS core protein; envelope protein; matrix protein;

SOURCE nonstructural protein.
 Hepatitis C virus isolated from human plasma, cDNA to genomic RN

A. ORGANISM Hepatitis C virus
 Viridae; ss-RNA enveloped viruses; Positive strand RNA virus;
 5 Togaviridae incertae sedis.
 REFERENCE 1 (bases 1 to 9416)
 AUTHORS Takamizawa, A., Mori, C., Manabe, S., Murakami, S., Fujita, J.,
 Onishi, E., Andoh, T., Yoshida, I. and Okayama, H.
 TITLE The structure and organization of the Hepatitis C virus genome
 10 isolated from human carriers
 JOURNAL J. Virol. 65, 1105-1113 (1991)
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 PALSTGLIHLHQNIVDVQYLYGIGSAVVSFAIKWEYVLLLFLLLADARVCACLWMLL
 IAQAEAALENLVVLSASVAGAHGILSFLVFFGAQWYIKGRVPGATYALYGVPWPLLL
 45 LLLALPPRAYAMDREMAASCGBAVFVGLVLLTSPYYKVFLARLIWLDQYFTTREAAD
 LHWVIFPLNARGGRDAIILLMCAVHPELIFDITKLLIAILGPLMVLQAGITRVYPFVR
 AQDLIHACMLVRKVAGGHYVQMAFMKLQALTGTYIYNHLTPLRDWPRAGLRDLAVAVE
 PVVFSDMETKIIITWGADTAACGDIILGLFVSARRGKEILLGPADSLEGRQLRLAPIT
 AYSQQTRGLLGCIIITSLTGRDKNQVGEVQVVSTATQSFLATGVNGVCWTVYWGAGSK

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AIPIEAIRGGRHLIFCHSKKKCDELAAKLSGLGINAVAYYRGLEDVSVIPTIGDVVVVA
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DHLEFWESVFTGLTHIDAHFLSQTQAGDNFPYLVAYGATVCARAGAPPPSWDMWKD
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ICORHVGPGEAGVQWMNRLIAFASRGNHVSPTHYVPESDAAARVTQILSSLTITQLLK
RLHQWINEDCSTPCSGSWLRDVWDWICTVLTDFKTWLGSKLLPQLPGVPPFSCQRGYK
GVVRGDSGIMQTTCPGCAQITGHVKNBSMRIVGPKTCSNTWHGTFFINAYTTGPTPSP
35
APNYSRALWRVAAEEYVEVTRVGDFHYVTGMTT DNVKCPCQVPAPEFFSEVDGVRLHR
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ARQSPPSLASSASQLSAPSLKATCTTHHVSPDADLIEANLLWRQEMGBNITRVESEN
KVVVLDSFDPLRAEEDEREVSUPAEILRKSKKFRAMP IWARPDYNPPLLESWKDPDY
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45
ATALPDQASDDGDKGSDVESYSSMPFLEGEFGDPDLSGGSWSTVSEASEDVVCCSMS
YTWTGALITPCAAEESKLPINALSNSLLRHNMUVATTSGSAGLRQKKVTFDRLEQVLD
DEQRDVLKEMKAKASTVKAKLLSVEEACKLTPPHSAKSKFGYAKDVRLSSKAVNHI
HSVWKDLLEDVTPTIDTTIMAKNEVFCVQPEKGBRKPARLIVFPDLGVRVCEKMALYD
VVSTLPQVVMGSSYGFQYSPGQRVEFLVNTWKSCKNPMGFSYDTRCFDSTVTENDIRV

EEGIYGCCLAPEARDAIKSLTERLYIGGPLTNSKGQNCQYRRCRASGVLTTSCGNTL
 TCYLKASAAQRAAKLQDCTMLVNGDDLUVICESAGTGEDAASLRVFTEAMTRYSAAPP
 DPPQPEYDLELITSCSSNVSVANDASGKRVVYLTRDPTPLARAAWETARHTPVNSWL
 GNIIMYAPTLWARMILMTHFFSILLAGEQLEKALDCQIYGACYSIEPLDLPQIIERLH
 GLQAFSLHSYSPGEINRVASCLRKLGVPPLRVWRHRARSVRARLLSQGGRAATCGKYL
 FNWQVKTKLKLTPIPAASRLDLGWFVAGYSGGDIYHSLSRARPRWFMLCLLLSVGV

	mat_peptide	GIYLLPNR"
15		336..677
		/product="core protein"
	mat_peptide	/codon_start=1
		678..905
		/product="matrix protein"
		/codon_start=1
20	mat_peptide	906..1499
		/product="envelope protein"
		/codon_start=1
	mat_peptide	1500..2519
		/product="non-structural protein"
25		/label=NS1
		/codon_start=1
	mat_peptide	2520..3350
		/product="non-structural protein"
		/label=NS2
30		/codon_start=1
	mat_peptide	3351..5177
		/product="non-structural protein"
		/label=NS3
		/codon_start=1
35	mat_peptide	5178..5918
		/product="non-structural protein"
		/label=NS4a
		/codon_start=1
	mat_peptide	5919..6371
40		/product="non-structural protein"
		/label=NS4b
		/codon_start=1
	mat_peptide	6372..9362
		/product="non-structural protein"
45		/codon_start=1

BASE COUNT 1905 a 2825 c 2679 g 2007 t
 ORIGIN

Initial Score	=	223	Optimized Score	=	223	Significance	=	40.30
Residue Identity	=	92%	Matches	=	223	Mismatches	=	17
Gaps	=	0	Conservative Substitutions	=			=	0

X 10 20 30 40 50 60
 ATGAGCAGCATTCCCAACCTCAAGAGAAAACCAACCTAACACCAACCGTCGCCCCACAGGA


```

XX
CC *source: isolate=Patient 1: Hepatitis C virus, JK1;
XX
FH Key Location/Qualifiers
FB
FT 5'UTR 1..324
FT CDS 325..2672
FT /gene="JK1" /partial
FT mat_peptide 325..897
FT /product="core"
FT mat_peptide 898..1473
FT /standard_name="E1" /product="envelope 1"
FT mat_peptide 1473..2523
FT /standard_name="NS1/E2" /note="boundary between
FT NS1 and E2"
FT mat_peptide 2524..2672
FT /standard_name="NS2" /product="non-structural 2"
XX
SQ Sequence 2672 BP; 504 A; 797 C; 778 G; 593 T; 0 other;
20
Initial Score = 222 Optimized Score = 222 Significance = 39.96
Residue Identity = 92% Matches = 222 Mismatches = 18
Gaps = 0 Conservative Substitutions = 0
25 X 10 20 30 40 50 60
ATGAGCAGCATTCCCAAACTCAAAGAAAACCAAAACGTACACCAACCGTCGCCCACAGGA
|||||
ACCGTCACCATGAGCACAATCCCAAACTCAAAGAAAACCAAAACGTACACCAACCGTCGCCCACAGGA
320 X 330 340 350 360 370 380
30 70 80 90 100 110 120 130
CGTCAAGTTCCTGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTGGCGCGCAGGGGCCCTAGATTGGG
|||||
CGTCAAGTTCCTGGGTGGCGGTGAGATCGTTGGTGGAGTTTACCTGTTGGCGCGCAGGGGCCCTAGATTGGG
35 390 400 410 420 430 440 450
140 150 160 170 180 190 200
TGTGCGCGCGACGAGGAGAGACTTCCGAGCGGTGCGAACCTCGAGGTAGACGTACGCTATCCCCAAGGACAG
|||||
40 TGTGCGCGCGACGAGGAGAGACTTCCGAGCGGTGCGAACCTCGTGGAGGCGACACCTATCCGCAAGGCTCG
460 470 480 490 500 510 520 530
210 220 230 240
TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
45 | |||||
CCAGCCCGAGGGCAGGACCTGGGCTCAGCCCGGGTACCCTTGGC
540 550 560 X 570

```

758WORTMAN-616-FIG1 (1-240)

HPCVJK1 Hepatitis C virus (JK1) core, E1, NS1/E2 and NS2 g.

LOCUS HPCVJK1 2672 bp ss-mRNA

URL 05-DEC-1991

DEFINITION Hepatitis C virus (JK1) core, E1, NS1/E2 and NS2 genes

ACCESSION X61591
 KEYWORDS NS1 protein; NS1/E2 protein; NS2 protein; core protein; envelope glycoprotein E1.
 SOURCE Hepatitis C virus RNA.
 ORGANISM Hepatitis C virus
 Viridae; ss-RNA enveloped viruses; Positive strand RNA virus; Togaviridae incertae sedis.
 REFERENCE 1 (bases 1 to 2672)
 AUTHORS Honda, M., Kaneko, S., Masashi, U., Kobayashi, K. and Murakami, S.
 TITLE Sequence analysis of putative structural regions of Hepatitis C Virus isolated from 5 Japanese patients
 JOURNAL Unpublished (1991)
 STANDARD full automatic
 COMMENT *source: isolate=Patient 1: Hepatitis C virus, JK1;
 15 From EMBL entry HCVJK1; dated 28-OCT-1991.

FEATURES Location/Qualifiers
 5' UTR 1..324
 mat_peptide 325..897
 20 CDS /product="core"
 325..2672
 /partial
 /gene="JK1"
 /codon_start=1
 mat_peptide 898..1473
 25 /standard_name="E1"
 /product="envelope 1"
 mat_peptide 1473..2523
 /standard_name="NS1/E2"
 /note="boundary between NS1 and E2"
 30 mat_peptide 2524..2672
 /standard_name="NS2"
 /product="non-structural 2"

BASE COUNT 504 a 797 c 778 g 593 t

ORIGIN

35

Initial Score = 222 Optimized Score = 222 Significance = 39.96
 Residue Identity = 92% Matches = 222 Mismatches = 18
 Gaps = 0 Conservative Substitutions = 0

40 X 10 20 30 40 50 60
 ATGAGCAGCATTCCCAACCTCAAGAGAAAACCAACCTAACACCAACCGTCGCCACAGGA
 ACCGTGCACCATGAGCACAATCCCAACCTCAAGAGAAAACCAACCTAACACCAACCGTCGCCACAGGA
 320 X 330 340 350 360 370 380
 45 70 80 90 100 110 120 130
 CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTTCGCCGCGCAGGGGCCCTAGATTGGG
 CGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTTCGCCGCGCAGGGGCCCTAGATTGGG
 50 390 400 410 420 430 440 450
 140 150 160 170 180 190 200
 TGTGCGCGCAGCAGGGAAGACTTCCBAGCGGTGCGAACCTCGAGGTAGACGTGAGCCTATCCCCAAGGCACG

FT /standard_name="NS1/E2" /note="boundary between
 FT NS1 and E2"
 FT mat_peptide 2524..2672
 FT /standard_name="NS2" /product="non-structural 2"
 XE
 SQ Sequence 2672 BP; 504 A; 797 C; 778 G; 593 T; 0 other;

Initial Score = 222 Optimized Score = 222 Significance = 39.96
 Residue Identity = 92% Matches = 222 Mismatches = 18
 Gaps = 0 Conservative Substitutions = 0

```

      X      10      20      30      40      50      60
      ATGAGCACGATTCCCAACCTCAAGGAAAAACCAACGTAACACCAACCGTCGCCACAGGA
15 ACCGTGCACCATGAGCACAATCCCAACCTCAAGGAAAAACCAACGTAACACCAACCGTCGCCACAGGA
   320   X 330   340   350   360   370   380
      70      80      90     100     110     120     130
      CGTCAAGTTCCCGGGTGGCGGTGATCGTTGGTGGAGTTTACTTGTTCGCCGCGAGGGGCCCTAGATTGGG
20 TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT
      CGTCAAGTTCCCGGGTGGTGGTATCGTTGGTGGAGTTTACTTGTTCGCCGCGAGGGGCCCTAGATTGGG
   390   400   410   420   430   440   450
      140     150     160     170     180     190     200
25 TGTGCGCGCGACGAGGAGACTTCCGAGCGGTGCGAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGCTCG
      TGTGCGCGCGACGAGGAGACTTCCGAGCGGTGCGAACCTCGTGAAGGCGACAACTATCCCCAAGGCTCG
   460   470   480   490   500   510   520   530
30 210     220     230     240
      TCGGCCCGAGGGCAGGACCTGGGCTCAGCCCAGG
      TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT
      CCAGCCCGAGGGCAGGACCTGGGCTCAGCCCAGGTTACCTTGGC
   540   550   560   X 570
35

```

9. WORTMAN-616-F101 (1-240)
 HPCCORE1 Hepatitis C virus core protein gene, partial cds.
 LOCUS HPCCORE1 534 bp ss-RNA VRL 17-OCT-1991
 DEFINITION Hepatitis C virus core protein gene, partial cds.
 ACCESSION M61718
 KEYWORDS core protein; polyprotein.
 SOURCE Hepatitis C virus, cDNA to mRNA.
 45ORGANISM Hepatitis C virus
 Viridae; ss-RNA enveloped viruses; Positive strand RNA virus;
 Togaviridae incertae sedis.
 REFERENCE 1 (bases 1 to 534)
 AUTHORS Fuchs, K., Motz, M., Schreier, E., Zachoval, R., Deinhardt, F. and
 50 Roggendorf, M.
 TITLE Characterization of nucleotide sequences from european hepatitis
 C virus isolates.
 JOURNAL Gene 103, 163-169 (1991).
 STANDARD simple staff_entry

FEATURES
CDS

Location/Qualifiers

(1..)534

/note="/product='core protein' /codon_start=1 /partial"

BASE COUNT
ORIGIN

88 a 168 c 167 g 111 t

Initial Score = 221 Optimized Score = 221 Significance = 39.61
Residue Identity = 99% Matches = 221 Mismatches = 1
Gaps = 0 Conservative Substitutions = 0

```

10      10      20      30      40      50      60      70      80
GATTCCCAAAACCTCAAGAGAAAACCAAAACGTAACACCAACCGTCGCCACAGGACGTCAGATTCCCGGGTGG
|||||
CCTCAAGAGAAAACCAAAACGTAACACCAACCGTCGCCACAGGACGTCAGATTCCCGGGTGG
15      X      10      20      30      40      50      60
      90      100     110     120     130     140     150
CGGTCAGATCGTTGGTGGAGTTTACTTGTTCGCCGCGCAGGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAA
|||||
20 CGGTCAGATCGTTGGTGGAGTTTACTTGTTCGCCGCGCAGGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAA
      70      80      90     100     110     120     130
      160     170     180     190     200     210     220
BACTTCCGAGCGGTGCGAACCTCGAGGTAGACGTGAGCCTATCCCAAGGCACGTGCGCCCGAGGGCAGGAC
25 -H-|||||
BACTTCCGAGCGGTGCGAACCTCGAGGTAGACGTGAGCCTATCCCAAGGCACGTGCGCTCGAGGGCAGGAC
      140     150     160     170     180     190     200
      230     240
30 CTGGGCTCAGCCCGGG
|||||
CTGGGCTCAGCCCGGGTATCCTTGGC
      210     220 X      230

```

35
10. WORTMAN-616-F101 (1-240)
HCVJFK1G Hepatitis C virus core, E1, NS1/E2, NS2, NS3, NS4a

ID HCVJFK1G standard; RNA; VRL; 9408 BP.
X0
AC X61596;
XX
DT 28-OCT-1991 (Rel. 29, Last updated; Version 1)
DT 28-OCT-1991 (Rel. 29, Created)
X5
DE Hepatitis C virus core, E1, NS1/E2, NS2, NS3, NS4a, NS4b and NS5
DE genes
XX
KW core protein; envelope glycoprotein E1; NS1 protein;
B0 NS1/E2 protein; NS2 protein; NS3 protein; NS4a protein;
KW NS4b protein; NS5 protein.
XX
OS Hepatitis C virus
OC Viridae; ss-RNA enveloped viruses; Positive strand RNA viruses;


```

OC Togaviridae.
XX
RN [1]
RA Honda M.
RE ;
RL Submitted (25-SEP-1991) on tape to the EMBL Data Library by:
RL M. Honda, First Dept of Internal Medicine, Kanazawa University,
RL takara-machi 13-1, Kanazawa, JAPAN
XX
RE [2]
RA Honda M., Kaneko S., Masashi U., Kobayashi K., Murakami S.;
RT "A whole genome of Hepatitis C Virus cDNA was isolated from a
RT single patient's liver tissue RNA";
RL Unpublished.
XX
CC *source: isolate=patient 1: Hepatitis C virus, JK1-full;
XX
FH Key Location/Qualifiers
FH
20 5' UTR 1..324
FT CDS 325..9357
FT /gene="JK1-full"
FT mat_peptide 325..897
FT /product="core"
25 mat_peptide 898..1473
FT /standard_name="E1" /product="envelope 1"
FT mat_peptide 1474..2523
FT /standard_name="NS1 /E2" /note="boundary between
FT NS1 and E2"
20 mat_peptide 2524..3342
FT /standard_name="NS2" /product="non-structural 2"
FT mat_peptide 3343..5169
FT /standard_name="NS3" /product="non-structural 3"
FT mat_peptide 5170..5910
25 /standard_name="NS4a" /product="non-structural 4a"
FT mat_peptide 5911..6363
FT /standard_name="NS4b" /product="non-structural 4b"
FT mat_peptide 6364..9354
FT /standard_name="NS5" /product="non-structural 5"
20 3' UTR 9355..9408
XX
SQ Sequence 9408 BP; 1886 A; 2826 C; 2691 G; 2005 T; 0 other;

Alignment Statistics:
Residue Identity = 92% Optimized Score = 221 Significance = 39.01
Matches = 221 Mismatches = 19
Gaps = 0 Conservative Substitutions = 0

Sequence Alignment:
X 10 20 30 40 50 60
ATGAGCAGCAGATTCCCAACCTCAAGAGAAAAACCAACGTAACACCAACCGTCGCCCCACAGGA
50
ACCGTGACCATGAGCAGCAATCCTAACCTCAAGAGAAAAACCAACGTAACACCAACCGTCGCCCCACAGGA
320 X 330 340 350 360 370 380
70 80 90 100 110 120 130

```



```

CGTCAAGTTCCCGGGTGGCGGGTCAGATCGTTGGTGGAGTTTACTTGTGTGCCGCGCAGGGGGCCCTAGATTGGG
|||||
CGTCAAGTTCCCGGGCGGGTGGTCAGATCGTTGGTGGAGTTTACCTGTGTGCCGCGCAGGGGGCCCCAGGTTGGG
390      400      410      420      430      440      450

5      140      150      160      170      180      190      200
TGTGCGCGCGACGAGGAAGACTTCCGAGCGGGTCGCGACCTCGAGGTAGACGTACGCCCTATCCCCAAGGGCAGG
|||||
TGTGCGCGCGACCGAGGAAGACTTCCGAGCGGGTCGCAACCTCGTGGAAAGGGCAGCAACCTATCCCCAAGGGCTCG
10460    470      480      490      500      510      520      530

      210      220      230      240
TCGGCCCGAGGGGACAGGACCTGGGCTCAGCCCGGG
|||||
15 CCAGCCCGAGGGGACAGGACCTGGGCTCAGCCCGGGTACCCTTGGG
      540      550      560      X      570

```


> 0 <
01.10 IntelliGenetics
> 0 <

FastDB -- Fast Pairwise Comparison of Sequences
Release 5.4

Results file wortman-616-fig1b-a-geneseq.res made by maryh on Wed 25 Mar 92
13:29:06-PST.
10.

Query sequence being compared: WORTMAN-616-FIG1 (1-240)
Number of sequences searched: 14140
Number of scores above cutoff: 4629
15

Results of the initial comparison of WORTMAN-616-FIG1 (1-240) with:
Data bank : A-GeneSeq 5, all entries

10000--
20
N --*
U 5000--
M --
P --
E25 --*
R --**
*
O
F 1000--*
30
S
E 500--
Q --*
U --*
E35
N
C
E
S 100--**
40
50
45 --*
10
50
5
--

5 0

SCORE	01	1	81	116	1	124	32	39	47	55	63	71
STDEV	0	1	3	5	7	9						

PARAMETERS

Similarity matrix	Unitary	K-tuple	2
Translation Frame	1		
Mismatch penalty	1	Joining penalty	20
Gap penalty	1.00	Window size	32
Gap size penalty	0.05		
Cutoff score	2		
Randomization group	0		
Initial scores to save	20	Alignments to save	10
Optimized scores to save	20	Display context	10

SEARCH STATISTICS

Scores:	Mean	Median	Standard Deviation
	3	4	2.28

Times:	CPU	Total Elapsed
30	00:00:30.06	00:01:01.00

Number of residues:	2168208
Number of sequences searched:	14140
Number of scores above cutoff:	4629

35
Cut-off raised to 3.
Cut-off raised to 4.
Cut-off raised to 5.

THE scores below are sorted by initial score.
Significance is calculated based on initial score.

A 100% identical sequence to the query sequence was not found.

45
The list of best scores is:

Sequence Name	Description	Length	Init. Score	Opt. Score	Sig. Fra
1. R13343	P1 HCV antigen (1-75)	75	71	71	29.83
2. R13558	HCV c protein immunodominant	9	71	71	29.83
3. R11374	Hepatitis C virus 17 isolate	154	71	71	29.83

4. R08124 Hepatitis C virus putative po 2955 71 71 29.83

5. R12600 PT-NANBH viral structural and 603 70 70 29.40

**** 27 standard deviations above mean ****

6. R12597 PT-NANBH viral structural prot 66 65 65 27.20

**** 26 standard deviations above mean ****

7. R12596 Antigenic portion of PT-NANBH 278 63 63 26.32

**** 16 standard deviations above mean ****

8. R13346 P35 HCV antigen (35-75). 42 40 40 16.23

**** 3 standard deviations above mean ****

9. P70247 AA sequence (VII) of a polype 191 12 15 3.95

10. P80627 AA sequence (VII) of polypept 191 12 15 3.95

11. P70244 AA sequence (IV) of a polypep 194 12 15 3.95

12. P80624 AA sequence (IV) of polypepti 194 12 15 3.95

13. P70243 AA sequence (III) of a polype 197 12 15 3.95

14. P80478 Sequence of a polypeptide hav 197 12 15 3.95

15. P70245 AA sequence (V) of a polypept 202 12 15 3.95

16. P80625 AA sequence (V) of polypeptid 202 12 15 3.95

17. P70248 AA sequence (VIII) of a polyp 224 12 15 3.95

18. P80628 AA sequence (VIII) of polypep 224 12 15 3.95

19. P70246 AA sequence (VI) of a polypep 236 12 15 3.95

20. P80626 AA sequence (VI) of polypepti 236 12 15 3.95

Query sequence being compared: WORTMAN-616-FIG1 (1-240)

Number of sequences optimized: 4629

25

Results of the optimized comparison of WORTMAN-616-FIG1 (1-240) with:
Data bank: A-GeneSeq 5, all entries

1000-
30: -
N -
U 500-
M -
B * *
E35 -

* * * *
* * * *
* * * *
* * * *

R
 O
 F
 100-40
 S
 E
 50-
 U
 E45
 N
 C
 S
 10-
 50

R

5 0-----

STDEV-3	1	1	1	1	1	1	1	1	1	1
SCORE 8	-2	-1	0	1	2	3	4	5	6	21
	9	11	12	13	15	16	18	20		

PARAMETERS

Similarity matrix	Unitary	K-tuple	2
Translation Frame	1		
Mismatch penalty	1	Joining penalty	20
Gap penalty	1.00	Window size	32
Gap size penalty	0.05		
Cutoff score	2		
Randomization group	0		
Initial scores to save	20	Alignments to save	10
Optimized scores to save	20	Display context	10

SEARCH STATISTICS

Scores:	Mean	Median	Standard Deviation
	12	15	1.60

Times:	CPU	Total Elapsed
30	00:01:37.92	00:03:17.00

Number of residues:	1504761
Number of sequences optimized:	4629

35
The scores below are sorted by optimized score.
Significance is calculated based on optimized score.

A 100% identical sequence to the query sequence was not found.
40

The list of best scores is:

Sequence Name	Description	Length	Init. Score	Opt. Score	Sig. Fra
45					
**** 36 standard deviations above mean ****					
1. R13343	P1 HCV antigen (1-75).	75	71	71	36.85
2. R13558	HCV core protein immunodominant	119	71	71	36.85
3. R11274	Hepatitis C virus J7 isolate	154	71	71	36.85
4. R08124	Hepatitis C virus putative po	2955	71	71	36.85
5. R12600	RT-NONBH viral structural and	603	70	70	36.22

1
1
6. R12597 **** 33 standard deviations above mean ****
PT-NANB viral structural prot 66 65 65 33.10

**** 31 standard deviations above mean ****
Antigenic portion of PT-NANB 278 63 63 31.85

**** 17 standard deviations above mean ****
P35 HCV antigen (35-75). 42 40 40 17.49

**** 6 standard deviations above mean ****
Sequence B encoded by a porti 183 9 22 6.25

**** 5 standard deviations above mean ****
CD4-specific CDR-grafted heav 464 6 21 5.62

**** 4 standard deviations above mean ****
Herpes Simplex virus-1 gC pro 509 7 20 5.00

Pseudorabies virus gp63 prote 350 8 20 5.00

R gene product of cDNA to HIV 105 5 19 4.37

Protein encoded by sequence w 944 6 19 4.37

IL-2R beta chain. 551 5 19 4.37

Sequence encoded by pol gene 1056 5 19 4.37

Plasmid pENV(80)-GAG-15. 291 6 19 4.37

Sequence encoded by human tra 442 6 19 4.37

BanI methylase. 428 8 19 4.37

Sequence of human estrogen-re 433 5 19 4.37

1. WORTMAN-616-FIB1 (1-240)
R13343 P1 HCV antigen (1-75).

2B R13343 standard; Protein; 75 AA.

AC R13343;

DT 23-OCT-1991 (first entry)

DE P1 HCV antigen (1-75).

KW C100-3; hepatitis C virus; immunoassay; epitope.

QQ Synthetic.

PN AU9068390-A.

PD 27-JUN-1991.

PF 21-DEC-1990; 068390.

PR 22-DEC-1989; US-456162.

QS 27-NOV-1990; US-610180.

PA (ABBO) ABBOTT LABORATORIES.

DR WPI; 91-238393/33.

DR N-PSDB; Q13146.

PT Immunological assays for hepatitis C virus antibody - by using polypeptide(s) contg. epitope(s) of hepatitis C virus antigens

PS Claim 10; Page 48; 62pp; English.

CC The polypeptide may be prepared by solid phase synthesis fragment

CC coupling or using recombinant technology.

CC The assay has increased sensitivity and is more specific than

SS assays using the polypeptide C100-3 (EP-318216).

CC See also Q13146-48 and R13343-65.

SD Sequence 75 AA;

SD 2 A; 15 R; 4 N; 1 D; 0 B; 0 C; 5 Q; 2 E; 0 Z; 9 G; 0 H;

SD 2 I; 3 L; 7 K; 1 M; 1 F; 10 P; 3 S; 5 T; 0 W; 1 Y; 4 V;

SD

Initial Score = 71 Optimized Score = 71 Significance = 36.85

Residue Identity = 95% Matches = 71 Mismatches = 3

Gaps = 0 Conservative Substitutions = 0

Translation Frame = 1

X 10 20 30 40 50 60 70
 MSTIPKPKRKTKRNTNRRPQDVKFPGGGGI VGGVYLLPRRGPRLGVRATRKTSERSQPRGRRQPIPKARRPE
 III III I
 MSTNPKPKQKKNKRNTRRPQDVKFPGGGGI VGGVYLLPRRGPRLGVRATRKTSERSQPRGRRQPIPKARRPE
 5 X 10 20 30 40 50 60 70
 X
 GR
 II
 10 GRT
 X

2. WORTMAN-616-FIG1 (1-240)
 15R13558 HCV core protein immunodominant region.

ID R13558 standard; Protein; 119 AA.
 AC R13558;
 DT 28-OCT-1991; (first entry)
 BB HCV core protein immunodominant region.
 KW Hepatitis C virus; non-A non-B hepatitis virus; diagnosis;
 KW C-100 protein; core protein; vaccines; NANBHV.
 OS Synthetic.
 FH Key Location/Qualifiers
 ES Peptide 1..61
 FT /label= VIIIE
 FT Peptide 59..119
 FT /label= IXE
 PN EP-442394-A.
 BD 21-AUG-1991.
 PF 08-FEB-1991; 101787.
 PR 16-FEB-1990; US-481348.
 PR 16-APR-1990; US-510153.
 PR 26-JUL-1990; US-558799.
 BB (UNBI-) UNITED BIOMED INC.
 PI Wang CY;
 DR WPI; 91-247104/34.
 PT New synthetic peptide(s) from immuno-dominant regions of virus -
 PT for diagnosis of hepatitis C virus and non -A, -B hepatitis
 RU infection, esp. using enzyme-linked immuno-sorbent assay
 RS Disclosure; Page 16; 93pp; English.
 CC In selecting regions of the HCV protein for epitope analysis,
 CC peptides in the 40 mer size range with amino acid sequences covering
 CC the complete HCV C-100 protein and the core protein were synthesised.
 CC These were tested for their reactivity with serum from a patient
 CC positively diagnosed with HCV infection. The indicated
 CC two overlapping peptides from the HCV core protein region
 CC were found to have specific immunoreactivity with the positive
 CC control serum. The peptides may be used in highly sensitive and
 BB accurate methods for the early detection of antibodies to HCV in
 CC body fluids and the diagnosis of NANBHV infection. Because of
 CC their high immunoreactivity, the peptides are also useful in
 CC stimulating prodn. of antibodies to HCV and in vaccines to prevent
 CC HCV or NANBHV infection.

CC See also R13557 for C-100 protein immunodominant peptides.

SQ Sequence 119 AA;

SQ 3 A; 22 R; 4 N; 2 D; 0 B; 1 C; 7 Q; 3 E; 0 Z; 16 G; 0 H;

SQ 3 I; 7 L; 6 K; 0 M; 1 F; 17 P; 7 S; 7 T; 5 W; 3 Y; 5 V;

Initial Score = 71 Optimized Score = 71 Significance = 36.85
Residue Identity = 97% Matches = 71 Mismatches = 2
Gaps = 0 Conservative Substitutions = 0
Translation Frame = 1

10 X 10 20 30 40 50 60 70
MSTIPKPKRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRGPRLGVRATRKTSERSQPRGRQPIPKARRPE
STIPKPKRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRGPRLGVRATRKTSERSQPRGRQPIPKVRRPE
15 X 10 20 30 40 50 60 70
X
GR
II
20 GRTWAGPGVPWP
X 80

3. WORTMAN-616-FIG1 (1-240)

25R11274 Hepatitis C virus J7 isolate C/E domain polypeptid

ID R11274 standard; Protein; 154 AA.
AC R11274;
DT 30-MAY-1991 (first entry)
BB Hepatitis C virus J7 isolate C/E domain polypeptide prod.
KW Hepatitis C virus; HCV-J1; HCV-J7; vaccines; NANBH.
OS Hepatitis C virus.
FH Key Location/Qualifiers
FT Misc_difference 8..8
BS /label= Gln, Arg
FT Misc_difference 25..25
FT /label= Pro, Leu
FT Misc_difference 91..91
FT /label= Leu, OTHER
BB /note= "OTHER= termination of sequence"
FT Misc_difference 110..110
FT /label= Asn, Thr
FT Misc_difference 130..130
FT /label= Phe, Leu
BB EP-419182-A.
PD 27-MAR-1991.
PF 17-SEP-1990; 310149.
PR 15-SEP-1989; US-408045.
PR 21-DEC-1989; US-456142.
BB (CHIR-) CHIRON CORP.
PI Miyamura T, Saito T, Houghton M, Weiner AJ, Han J;
PI Kolberg JA, Chata T-A, Irvine BD;
DR WPI; 91-088781/13.
DR N-PSDB; Q11075.

PT New isolates J1 and J7 of hepatitis C virus - contg. specified
 PT DNA and amino acid sequences, used in diagnosis, recombinant
 PT protein prodn. and vaccine
 PS Disclosure; fig 1; 109pp; English.
 CB This polypeptide prod. is encoded by a fragment of the hepatitis
 CC C virus (HCV) J7 isolate C/E domain. This is one of the domains of
 CC the viral isolate exhibiting heterogeneity w.r.t the HCV1 isolate.
 CC The corresp. nucleotide sequence has an important potential use as
 CC a probe in diagnostic assays and vaccine development. Antibodies
 CC directed against it can be used for screening antiviral agents and
 CC for isolation of non-A, non-B hepatitis (NANBH).
 CC See also Q11076-79.
 SQ Sequence 154 AA;
 SQ 10 A; 23 R; 5 N; 4 D; 0 B; 1 C; 5 Q; 3 E; 0 Z; 23 G; 1 H;
 SQ 4 I; 12 L; 7 K; 2 M; 1 F; 18 P; 7 S; 0 T; 5 W; 4 Y; 6 V;
 SQ 5 Others;

Initial Score = 71 Optimized Score = 71 Significance = 36.85
 Residue Identity = 95% Matches = 71 Mismatches = 3
 Gaps = 0 Conservative Substitutions = 0
 Translation Frame = 1

	X	10	20	30	40	50	60	70
	MSTIPKPKRKTKRNTNRRPQDVKFGGGQIVGGVYLLPRRGPRLGVRATRKTSERSQPRGRRQPIPKARRPE							
25	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
	MSTNPKPKXRKTKRNTNRRPQDVKFXGGGQIVGGVYLLPRRGPRLGVRATRKTSERSQPRGRRQPIPKARRPE							
	X	10	20	30	40	50	60	70

X
 30 GR
 !!
 GRTWAGPGYPWP
 X 80

35:
 4. WORTMAN-616-FIG1 (1-240)
 R08124 Hepatitis C virus putative polyprotein.

ID R08124 standard; protein; 2955 AA.
 AQ R08124;
 DT 23-JAN-1991 (first entry)
 DE Hepatitis C virus putative polyprotein.
 KW Hepatitis C virus (HCV); antiviral agent.
 OS Hepatitis C virus.
 RB Key Location/Qualifiers
 FT Misc_difference 9..9
 FT /label=K or R
 FT Misc_difference 11..11
 FT /label=N or T
 EQ Misc_difference 176..176
 FT /label=I or T
 FT Misc_difference 334..334
 FT /label=M or V
 FT Misc_difference 603..603

FT /label=I or L
FT Misc_difference 848..848
FT /label=Y or N
FT Misc_difference 1114..1114
ES /label=P or S
FT Misc_difference 1117..1117
FT /label=S or T
FT Misc_difference 1276..1276
FT /label=P or L
FT Misc_difference 1454..1454
FT /label=C or Y
FT Misc_difference 1471..1471
FT /label=T or S
FT Misc_difference 1877..1877
ES /label=E or G
FT Misc_difference 1948..1948
FT /label=L or H
FT Misc_difference 1949..1949
FT /label=S or C
EQ Misc_difference 2021..2021
FT /label=V or G
FT Misc_difference 2349..2349
FT /label=T or S
FT Misc_difference 2385..2385
ES /label=Y or F
FT Misc_difference 2386..2386
FT /label=S or A
FT Misc_difference 2502..2502
FT /label=L or F
EQ Misc_difference 2690..2690
FT /label=R or G
FT Misc_difference 2921..2921
FT /label=R or G
PN EP-388232-A.
EQ 19-SEP-1990.
PF 16-MAR-1990; 302866.
PR 17-MAR-1989; US-325338.
PR 20-APR-1989; US-341334.
PR 18-MAY-1989; US-355002.
EQ (CHIR-) CHIRON CORP.
PI Houghton M, Choo QL, Kuo G;
DR WPI; 90-284418/38.
DR N-PSDB; Q05956.
PT Hepatitis C virus DNA - used for producing probes,
ES polypeptide(s), antibodies and anti-sense polynucleotide(s) for
PT diagnosis and therapy.
PS Disclosure; Fig 17; 83pp; English.
CC HCV cDNA libraries were constructed using pooled serum from a
CC chimpanzee with chronic HCV infection. A lambda gt11 library was
EQ screened with probes derived from previously isolated clones. The
CC ORF is derived from the overlapping clones b114a, ag30a, CA205a,
CC CA290a, CA216a, pi4a, CA167b, CA156e, CA84a, CA59a, K9-1, 26j, 13i,
CC 12f, 14i, 11b, 7f, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c,
CC 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, 15e, b5a and 16jh.

CC Polypeptide encoded by this sequence can be used to design probes
 CC for the detection of HCV nucleic acids, in screening programmes
 CC for antiviral agents and in preparing blood free of HCV. The
 CC sequence contains 188 (overlapping) peptides which are claimed as
 CC HCV epitopes.
 CC See also Q05955.
 SQ Sequence 2955 AA;
 SQ 270A; 168R; 86 N; 119D; 0 B; 101C; 89 Q; 114E; 0 Z; 246G; 68 H;
 SQ 127I; 295L; 95 K; 54 M; 84 F; 205P; 201S; 214T; 68 W; 94 Y; 236V;
 SQ 21 Others;

Initial Score = 71 Optimized Score = 71 Significance = 36.85
 Residue Identity = 95% Matches = 71 Mismatches = 3
 Gaps = 0 Conservative Substitutions = 0
 Translation Frame = 1

```

X      10      20      30      40      50      60      70
MSTIPKPKRKTKRNTNRRPDVKFPGGGQIVGGVYLLPRRGPRLGVRAKTSERSQPRGRQPIPKARRPE
111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111
20 MSTNRPKPKXKXKRNTNRRPDVKFPGGGQIVGGVYLLPRRGPRLGVRAKTSERSQPRGRQPIPKARRPE
X      10      20      30      40      50      60      70

-X-
GR
25 11
GRTWAGPGYPWP
X      80

```

530WORTMAN-616-FIG1 (1-240)

R12600 PT-NANBH viral structural and non-structural prote

ID R12600 standard; Protein; 603 AA.
 AC R12600;
 DS 17-SEP-1991 (first entry)
 DE PT-NANBH viral structural and non-structural proteins.
 KW post-transfusional non-A, non-B hepatitis; virus; vaccine; ss.
 OS Non-A, non-B hepatitis virus.
 PN GB2239245-A.
 RD 26-JUN-1991.
 RF 17-DEC-1990; 027250.
 PR 10-DEC-1989; GB-028562.
 PR 27-FEB-1990; GB-004414.
 PR 03-MAR-1990; GB-004814.
 RB 17-DEC-1990; GB-027250.
 RA (WELL) WELLCOME FOUNDATION LTD.
 PI Highfield PE, Rodgers BC, Tedder RS, Barbara JAJ;
 DR WPI; 91-187584/26.
 DR N-PSDB; Q12242.
 BM Post-transfusional non-A non-B hepatitis polypeptide(s) - and
 PT also DNA and antibodies used in diagnostic assays and in vaccines
 PS Claim 1; Page 03-97; 108pp; English.
 CC The sequence was deduced from a "structural/non-structural" coding
 CC region sequence isolated from serum of humans infected by the

CC See also Q12236-41.

50 ~~Séquence~~ 603 AA;

Initial Score = 70 Optimized Score = 70 Significance = 36.22

Residue Identity = 94% Matches = 70 Mismatches = 4

Gaps = 0: Conservative Substitutions = 0

Translation Frame=

X	10	20	30	40	50	60	70
---	----	----	----	----	----	----	----

MSTIPKPGRKTKRNTNRRPGDVKFPGGGQIVGGVYLLPRRGPRLGVRATRKTSERSQPRRRQPIPKARR

[illegible]

20 MSTNFKPQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPTLGVRATRKTSERSQPRRRQPINARG

X	10	20	30	40	50	60	70
---	----	----	----	----	----	----	----

[Faint handwritten notes at the bottom of the page]

X.

GR

25.11.1954

GRAWAQPGYFWP

X 50

630WORTMAN-616-FIG1 (1-240)

R12597 PT-NANB viral structural protein encoded by clone

ID R12597 standard; Protein; 66 AA.

AC R12597:

DB 06-SEP-1991 (first entry)

DE PT-NANB viral structural protein encoded by clone 164/137.

KW post-transfusional non-A, non-B hepatitis; virus; vaccine.

05 Non-A, non-B hepatitis virus.

PN GB2239245-A.

AD 26-JUN-1991.

PF 17-DEC-1990 027250

PR 18-DEC-1989; GB-028562.

PR 27-FEB-1990: GB-004414.

FR 03-MAR-1990 GB-004814

17-DEC-1990; GB-027250.

PA (WELL) WELLCOME FOUNDATION LTD.

FJ Highfield PE, Rodgers BC, Tedder RS, Barbara JAJ,

DR WFI; 91-187584/26.

DR N-PSDB; Q12237.

EM Post-transfusional non-A non-B hepatitis polypeptide(s) - and

PT also DNA and antibodies used in diagnostic assays and in vaccines

PS Claim 1; Page 71-72; 100pp; English.

CC The sequence was deduced from a structural coding region sequence

CC isolated from serum of humans infected by the PT-NANBH virus.

CC The polypeptide is an antigenic portion of the virus and will be
 CC useful in the development of vaccines for inducing immunity in man to
 CC PT-NANBH. The invention covers PT-NANBH viral polypeptides having
 CC an amino acid sequence at least 90 per cent homologous with the
 CC sequence given here, or antigenic fragments of such homologous
 CC sequences.

CC See also Q12236-8 and Q12240-Q12242.

SD Sequence 66 AA;

SD 1 A; 13 R; 3 N; 1 D; 0 B; 0 C; 5 Q; 1 E; 0 Z; 8 G; 0 H;
 SD 2 I; 3 L; 5 K; 1 M; 1 F; 9 P; 3 S; 5 T; 0 W; 1 Y; 4 V;

Initial Score = 65 Optimized Score = 65 Significance = 33.10
 Residue Identity = 98% Matches = 65 Mismatches = 1
 Gaps = 0 Conservative Substitutions = 0
 Translation Frame = 1

```

X      10      20      30      40      50      60      X 70
MSTIPKPKQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGRLGVRATRKTSERSQPRGRRQIPKARRPE
|||||
20 MSTNPKPKQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGRLGVRATRKTSERSQPRGRRQIP
X      10      20      30      40      50      60      X

```

GR

25

7. WORTMAN-616-FIG1 (1-240)

R12596 Antigenic portion of PT-NANB polypeptide encoded b

SD R12596 standard; Protein; 278 AA.

AC R12596;

DT 06-SEP-1991 (first entry)

DE Antigenic portion of PT-NANB polypeptide encoded by BR11.

KW post-transfusional non-A, non-B hepatitis; virus; vaccine.

GS Non-A, non-B hepatitis virus.

PN GB2239245-A.

PD 26-JUN-1991.

PF 17-DEC-1990; 027250.

PR 18-DEC-1989; GB-028562.

RO 27-FEB-1990; GB-004414.

RR 03-MAR-1990; GB-004814.

RS 17-DEC-1990; GB-027250.

SA (WELL) WELLCOME FOUNDATION LTD.

SI Highfield PE, Rodgers BC, Tedder RS, Barbara JAJ;

SO WPI; 91-187584/26.

DR N-PSDB; Q12238.

PT Post-transfusional non-A non-B hepatitis poly:peptide(s); and

PT also DNA and antibodies used in diagnostic assays and in vaccines

PS Claim 1; Page 56-58; 108pp; English.

SO The sequence was deduced from a structural coding region sequence

CC (BR11) isolated from serum of humans infected by the PT-NANBH virus

CC and screened with sera of patients with a high risk for PT-NANBH.

CC The polypeptide is an antigenic portion of the virus and will be

CC useful in the development of vaccines for inducing immunity in man to

CC PT-NANBH. The invention covers PT-NANBH viral polypeptides having
CC an amino acid sequence at least 90 per cent homologous with the
CC sequence given here, or antigenic fragments of such homologous
CC sequences.

CB See also Q12236-7 and Q12239-Q12242.

SC Sequence 278 AA;

SC 25 A; 32 R; 10 N; 10 D; 0 B; 0 C; 6 Q; 7 E; 0 Z; 30 G; 6 H;

SC 10 I; 23 L; 6 K; 4 M; 6 F; 22 P; 23 S; 16 T; 6 W; 8 Y; 20 V;

Initial Score = 63 Optimized Score = 63 Significance = 31.85
Residue Identity = 95% Matches = 63 Mismatches = 3
Gaps = 0 Conservative Substitutions = 0
Translation Frame = 1

```

15      10      20      30      40      50      60      70
MSTIPKPKRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATRKTSERSQPRRRQPIPKARRPE
||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
RKTKRNTNLRPQDVRFPGGGQIVGGVYLLPRRGPRLGVRATRKTSERSQPRRRQPIPKARQPE
X      10      20      30      40      50      60
20
X
GR
||
GRAWAQPGYPWP
25 X 70
```

8. WORTMAN-616-FIG1 (1-240)

R13346 P35 HCV antigen (35-75).

30
ID R13346 standard; Protein; 42 AA.
AC R13346;
DT 23-OCT-1991 (first entry)
DE P35 HCV antigen (35-75).
EO C100-3; hepatitis C virus; immunoassay; epitope.
OS Synthetic.
PN AU9068390-A
PD 27-JUN-1991.
PF 21-DEC-1990; 068390.
PQ 22-DEC-1989; US-456162.
PR 07-NOV-1990; US-610180.
PA (ABB0) ABBOTT LABORATORIES.
DR WPI; 91-238393/33.
PT Immunological assays for hepatitis C virus antibody - by using
PT polypeptide(s) contg. epitope(s) of hepatitis C virus antigens.
PS Claim 10; Page 48; 62pp; English.
CC The polypeptide may be prepared by solid phase synthesis fragment
CC coupling (pref.) or using recombinant technology.
CC The assay has increased sensitivity and is more specific than
SC assays using the polypeptide C100-3 (EP-318216).
CC See also Q13146-48 and R13343-65.
SC Sequence 42 AA;
SC 2 A; 12 R; 0 N; 0 D; 0 B; 0 C; 2 Q; 2 E; 0 Z; 4 G; 1 H;
SC 1 I; 3 L; 2 K; 0 M; 0 F; 6 P; 2 S; 3 T; 0 W; 1 Y; 1 V;

Initial Score = 40 Optimized Score = 40 Significance = 17.49
 Residue Identity = 97% Matches = 40 Mismatches = 1
 Gaps = 0 Conservative Substitutions = 0
 Translation Frame = 1

30 X 40 50 60 70 X
 FPGGGQIVGGVYLLPRGPRLGVRATRKTSERSQPRGRQPIPKARRPEGR
 |||||
 HYLLPRGPRLGVRATRKTSERSQPRGRQPIPKARRPEGR
 10 X 10 20 30 40

15

20

25

30

35

PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 1996

Application or Docket Number

8/819,857

CLAIMS AS FILED - PART I

(Column 1)

(Column 2)

FOR	NUMBER FILED	NUMBER EXTRA
BASIC FEE		
TOTAL CLAIMS	9 minus 20 =	
INDEPENDENT CLAIMS	1 minus 3 =	
MULTIPLE DEPENDENT CLAIM PRESENT		

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY

OR

OTHER THAN SMALL ENTITY

RATE	FEE		RATE	FEE
	385.00	OR		770.00
x\$11=		OR	x\$22=	
x40=		OR	x80=	
+130=		OR	+260=	
TOTAL		OR	TOTAL	170 ✓

CLAIMS AS AMENDED - PART II

(Column 1)

(Column 2)

(Column 3)

AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	*	Minus	20 =
Independent	*	Minus	3 =
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY

OR

OTHER THAN SMALL ENTITY

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=		OR	x\$22=	
x40=		OR	x80=	
+130=		OR	+260=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

(Column 1)

(Column 2)

(Column 3)

AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	*	Minus	=
Independent	*	Minus	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY

OR

OTHER THAN SMALL ENTITY

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=		OR	x\$22=	
x40=		OR	x80=	
+130=		OR	+260=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

(Column 1)

(Column 2)

(Column 3)

AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	*	Minus	=
Independent	*	Minus	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY

OR

OTHER THAN SMALL ENTITY

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=		OR	x\$22=	
x40=		OR	x80=	
+130=		OR	+260=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, enter 0 in column 3.
 * If the Highest Number Previously Paid For in this space is less than 20, enter 20.
 * If the Highest Number Previously Paid For in this space is less than 3, enter 3.
 * The Highest Number Previously Paid For (Total or Independent) is the highest number found in the appropriate box in column 1.

3	5	7
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ATTORNEY DOCKET NUMBER

2	6	7	3	4	3	2	6	6	
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PARENT FILING

**PARENT FILING
DATE**

01	08	76
08	27	50

FOREIGN FILING DATE	
MONTH	DAY
1	1
2	2
3	3
4	4
5	5
6	6
7	7
8	8
9	9
10	10
11	11
12	12

**MULTIPLE DEPENDENT CLAIM
FEE CALCULATION SHEET
(FOR USE WITH FORM PTO-875)**

SERIAL NO.

FILING DATE

APPLICANT(S)

CLAIMS

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
1	1					
2		1				
3		1				
4	1					
5	1					
6		1				
7		3				
8		3				
9		3				
10		3				
11		3				
12		3				
13	1					
14		1				
15		1				
16		1				
17	1					
18	1					
19		1				
20		3				
21		3				
22		3				
23		3				
24		3				
25		3				
26		3				
27		3				
28	1					
29		1				
30		1				
31		1				
32		1				
33		1				
34		1				
35		1				
36		1				
37		1				
38		3				
39		3				
40		3				
41						
42						
43						
44						
45						
46						
47						
48						
49						
50						
TOTAL IND.	17					
TOTAL DEP.	67					
TOTAL CLAIMS	74					

	*		*		*	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
51						
52						
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54						
55						
56						
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58						
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98						
99						
100						
TOTAL IND.						
TOTAL DEP.						
TOTAL CLAIMS						

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